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WO 03/027095 A1

(54) Title: SUBSTITUTED 3-PYRIDYL TETRAZOLES AS STEROID C17,20 LYASE INHIBITORS

(57) Abstract: The invention provides novel substituted 3-pyridyl tetrazoles and pharmaceutical compositions thereof. The invention also provides methods of use of these materials and pharmaceutical compositions as inhibitors of lyases, e.g., the 17a-hydroxylase-C17,20 enzyme. The invention further provides methods for the treatment of cancer in a subject, comprising administering a substituted 3-pyridyl tetrazoles of the invention or a pharmaceutical composition thereof to a subject. The cancer can be, e.g., prostate cancer or breast cancer.

APPLICATION FOR PATENT

SUBSTITUTED 3-PYRIDYL TETRAZOLES AS STEROID C17,20 LYASE INHIBITORS

Background of the Invention

Steroid biosynthesis begins in cells of the adrenal gland where the initial product in sterol biosynthesis, cholesterol, is converted into the adrenal steroid hormones aldosterone, hydrocortisone, and corticosterone by a series of P_{450} -mediated hydroxylation steps. The cholesterol side-chain cleavage activity that represents the first step in steroid hormone biosynthesis is a P_{450} -mediated oxidation and cleavage of a pair of adjacent methylene groups to two carbonyl fragments, pregnenolone and isocaprylaldehyde (see Walsh (1979) Enzymatic Reaction Mechanisms; W.H. Freeman and Company, pp. 474-77). Another critical set of enzymatic conversions in steroid metabolism is facilitated by 17-alpha-hydroxylase-17,20-lyase (CYP17, P_{450} 17). CYP17 is a bifunctional enzyme which possesses both a C17,20-lyase activity and a C17-hydroxylase activity. Significantly, these two alternative enzymatic activities of CYP17 result in the formation of critically different intermediates in steroid biosynthesis and each activity appear to be differentially and developmentally regulated (see e.g. l'Allemand et al. (2000) Eur. J. Clin. Invest. 30: 28-33).

The C17,20-lyase activity of CYP17 catalyzes the conversion of 17α–hydroxy-pregnenolone and 17α–hydroxy-progesterone to dehydroepiandrosterone (DHEA) and delta4-androstenedione (androstenedione) respectively. Both DHEA and androstenedione lyase products are key intermediates in the synthesis of not only the androgens testosterone and dihydrotestosterone (DHT), but also the estrogens 17-beta-estradiol and estrone. Indeed, adrenal and ovarian estrogens are the main sources of estrogens in postmenopausal women (see e.g. Harris et al. (1988) Br. J. Cancer 58: 493-6). In contrast, the C17-hydroxylase activity of CYP17 catalyzes the conversion of the common intermediate progesterone to 17-hydroxyprogesterone, a precursor of cortisol. Therefore the first activity of CYP17, the C17-hydroxylase activity, promotes the formation of glucocorticoids while the second activity of CYP17, the C17,20-lyase activity, promotes the formation of sex hormones - particularly androgens including testosterone as well as estrogens.

Prostate cancer is currently one of the most frequently diagnosed forms of cancer in men in the U.S. and Europe. Prostate cancer is typically androgen-dependent and, accordingly, the reduction in androgen production via surgical or pharmacological castration remains the major treatment option for this indication. However, complete rather than partial withdrawal of androgens may be more effective in treating prostate cancer (Labrie, F. *et al.*, *Prostate*, 1983, *4*, 579 and Crawford, E.D. *et al.*, *N. Engl. J. Med.*, 1989, *321*, 419). Pharmacological inhibition of CYP17 may be a promising alternative treatment to antiandrogens and LHRH agonists in that testicular, adrenal, and peripheral androgen biosynthesis would be reduced rather than only testicular androgen production (Njar V, *et al.*, *J. Med. Chem.*, 1998, *41*, 902). One such CYP17 inhibitor, the fungicide ketoconazole, has been used previously for prostate cancer treatment (Trachtenberg, J., *J. Urol.*, 1984, *132*, 61 and Williams, G. *et al.*, *Br. J. Urol.*, 1986, *58*, 45). However, this drug is a relatively non-selective inhibitor of cytochrome P450 (CYP) enzymes, has weak CYP17 activity, and has a number of notable side effects associated with it including liver damage (De Coster, R. *et al.*, *J. Steroid Biochem. Mol. Biol.*, 1996, *56*, 133 and Lake-Bakaar, G. *et al.*, *Br. Med. J.*, 1987, *294*, 419).

The importance of potent and selective inhibitors of CYP17 as potential prostate cancer treatments has been the subject of numerous studies and reviews (Njar, V. *et al.*, *Curr. Pharm. Design*, 1999, *5*, 163; Barrie, S.E. *et al.*, *Endocr. Relat. Cancer*, 1996, *3*, 25 and Jarman, M. *et al.*, *Nat. Prod. Rep.*, 1998, 495). Finasteride, a 5α-reductase inhibitor, is an approved treatment for benign prostatic hyperplasia (BPH), although it is only effective with patients exhibiting minimal disease. While finasteride reduces serum DHT levels, it increases testosterone levels, and may therefore be insufficient for prostate cancer treatment (Peters, D. H. *et al.*, *Drugs*, 1993, *46*, 177). Certain anti-androgenic steroids, for example, cyproterone acetate (17α-acetoxy-6-chloro-1α, 2α-methylene-4,6-pregnadiene-3,20-dione), have been tested as adjuvant treatments for prostate cancer. Many other steroids have been tested as hydroxylase/lyase inhibitors. See, for example, PCT Specification WO 92/00992 (Schering AG) which describes anti-androgenic steroids having a pyrazole or triazole ring fused to the A ring at the 2,3-position, or European specifications EP-A288053 and EP-A413270 (Merrell Dow) which propose 17β-cyclopropylamino-androst-5-en-3β-ol or -4-en-3-one and their derivatives.

In addition to the use of CYP17 inhibitors in the treatment of prostate cancer, a second potential indication would be for estrogen-dependent breast cancer. In postmenopausal patients with advanced breast cancer, treatment with high doses of ketoconazole resulted in suppression of both testosterone and estradiol levels, implicating CYP17 as a potential target for hormone therapy (Harris, A. L. *et al.*, *Br. J. Cancer*, 1988, *58*, 493).

Chemotherapy is usually not highly effective, and is not a practical option for most patients with prostate cancer because of the adverse side effects which are particularly detrimental in older patients. However, the majority of patients initially respond to hormone ablative therapy although they eventually relapse, as is typical with all cancer treatments (McGuire, in: *Hormones and Cancer*,. Iacobelli *et al.* Eds.; Raven Press, New York, 1980, *Vol. 15*, 337-344). Current treatment by orchidectomy or administration of gonadotropin-releasing hormone (GnRH) agonists results in reduced androgen production by the testis, but does not interfere with androgen synthesis by the adrenals. Following three months of treatment with a GnRH agonist, testosterone and DHT concentrations in the prostate remained at 25% and 10%, respectively, of pretreatment levels (Forti *et al.*, *J. Clin. Endocrinol. Metab.*, 1989, *68*, 461). Similarly, about 20% of castrated patients in relapse had significant levels of DHT in their prostatic tissue (Geller *et al.*, *J. Urol.*, 1984, *132*, 693). These findings suggest that the adrenals contribute precursor androgens to the prostate. This is supported by clinical studies of patients receiving combined treatment with either GnRH or orchidectomy and an anti-androgen, such as flutamide, to block the actions of androgens, including adrenal androgens. Such patients have increased progression-free survival time compared to patients treated with GnRH agonist or orchidectomy alone (Crawford *et al.*, *N. Engl. J. Med.*, 1989, *321*, 419 and Labrie *et al.*, *Cancer Suppl.*, 1993, *71*, 1059).

Although patients initially respond to endocrine therapy, they frequently relapse. It was reported recently that in 30% of recurring tumors of patients treated with endocrine therapy, high-level androgen receptor (AR) amplification was found (Visakorpi, *et al.*, *Nature Genetics*, 1995, *9*, 401). Also, flutamide tends to interact with mutant ARs, and stimulate prostatic cell growth. This suggests that AR amplification may facilitate tumor cell growth in low androgen concentrations. Thus, total androgen blockade as first line therapy may be more effective than conventional androgen deprivation by achieving maximum suppression of androgen concentrations which may also prevent AR

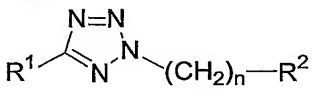
amplification. It is presently unclear whether sequential treatment with different agents can prolong the benefits of the initial therapy. This strategy has been found effective in breast cancer treatment. New agents which act by different mechanisms could produce second responses in a portion of relapsed patients. Although the percentage of patients who respond to second-line hormonal therapy may be relatively low, a substantial number of patients may benefit because of the high incidence of prostate cancer. Furthermore, there is the potential for developing more potent agents than current therapies, none of which are completely effective in blocking androgen effects.

The need exists for C17,20 lyase inhibitors that overcome the above-mentioned deficiencies.

Summary of the Invention

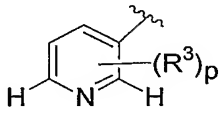
The invention provides substituted 3-pyridyl heterocyclic compounds which inhibit the lyase activity of enzymes, e.g., 17α-hydroxylase-C17,20 lyase.

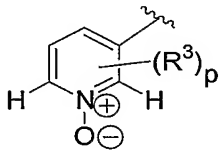
Compounds of the invention have the formula



in which n is 1 or 2;

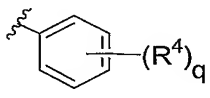
R^1 represents

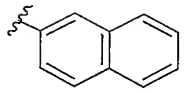
 in which R^3 is C_{1-4} alkyl or C_{3-5} cycloalkyl; and p is 0, 1, or 2; or

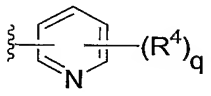
 , provided that R^2 is other than a pyridyl group;

and

R^2 represents

 in which R^4 represents C_{1-4} alkyl, C_{1-4} alkoxy, C_{3-6} cycloalkyl, halogen, NO_2, or CN; and q is 0, 1, or 2;


; or



.

Pharmaceutically acceptable salts of these materials are also within the scope of the invention.

The invention also provides pharmaceutical compositions for inhibiting lyase activity, comprising a compound of the invention and a pharmaceutically acceptable carrier.

The invention also provides methods for inhibiting lyases, comprising contacting the lyase with a compound of the invention. More particularly, the invention provides a method of inhibiting a 17α-hydroxylase-C17,20 lyase, comprising contacting a 17α-hydroxylase-C17,20 lyase with a compound of the invention.

The invention further provides methods for treating diseases which can benefit from an inhibition of a lyase enzyme. Exemplary diseases are lyase-associated diseases, e.g., diseases resulting from an excess of androgens or estrogens. For example, the invention provides a method for treating cancer in a subject, comprising administering to the subject a pharmaceutically effective amount of a compound of the invention, such that the cancer is treated.

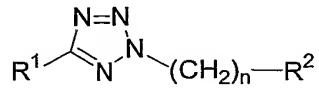
The method of treatment may be applied where the subject is equine, canine, feline, or a primate, in particular, a human.

The cancer may, for example, be prostate or breast cancer. Accordingly, a method for treating prostate cancer in a subject, comprises administering to the subject a therapeutically effective amount of a compound of the invention, such that the prostate cancer in the subject is treated. Similarly, a method for treating breast cancer in a subject comprises administering to the subject a therapeutically effective amount of a compound of the invention, such that the breast cancer in the subject is treated.

Detailed Description of the Invention

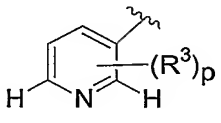
The invention is based at least in part on the discovery that substituted 3-pyridyl heterocyclic compounds inhibit the enzyme 17α-hydroxylase-C17,20 lyase.

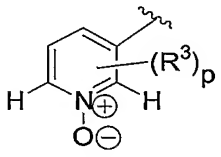
In a preferred embodiment, compounds of the invention have the formula



in which n is 1 or 2;

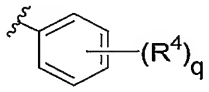
R^1 represents

 in which R^3 is C_{1-4} alkyl or C_{3-5} cycloalkyl; and p is 0, 1, or 2; or

 ;

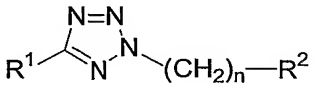
and

R^2 represents

 in which R^4 represents C_{1-4} alkyl, C_{1-4} alkoxy, halogen, NO_2, or CN; and q is 0, 1, or 2.

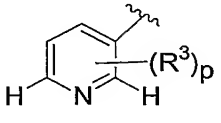
Pharmaceutically acceptable salts of these materials are also within the scope of the invention.

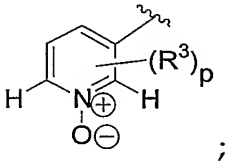
In a more preferred embodiment, compounds of the invention have the formula



in which n is 1;

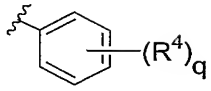
R^1 represents

 in which R^3 is C_{1-4} alkyl or C_{3-5} cycloalkyl; and p is 0, 1, or 2; or

 ;

and

R^2 represents

 in which R^4 represents C_{1-4} alkoxy, halogen, or NO_2, and q is 0, 1, or 2.

Pharmaceutically acceptable salts of these materials are also within the scope of the invention.

Definitions

For convenience, certain terms employed in the specification, examples, and appended claims are collected here.

The term "agonist" of an enzyme refers to a compound that binds to the enzyme and stimulates the action of the naturally occurring enzyme, or a compound which mimics the activity of the naturally occurring enzyme.

The term "antagonist" of an enzyme refers to a compound that binds to the enzyme and inhibits the action of the naturally occurring enzyme.

The term "analog" of a compound refers to a compound having a some structural similarity to a particular compound and having essentially the same type of biological activity as the compound.

The term "CYP17 substrate" includes any of the various steroid hormones acted upon by a CYP17 or a CYP17-like P_{450} enzyme. Examples include pregnenolone, progesterone and their 17α-hydroxylated forms. Pregnenolone is converted to DHEA via a CYP17 C17,20-lyase reaction, but is also subject to C17α-hydroxylation via the C17,20-lyase activity. Progesterone is converted to delta 4- androstenedione via a CYP17 C17,20-lyase reaction, but is also subject to C17 alpha-hydroxylation via the C17-hydroxylase activity to form 17-hydroxyl-progesterone, a precursor to hydrocortisone (i.e. cortisol).

The term "CYP17 metabolite" refers to any of the steroid hormones that are synthesized from a cholesterol precursor via a CYP17-mediated reaction, such as a C17-hydroxylase reaction or a C17,20-lyase reaction. Examples of CYP17 metabolites include the androgens, such as testosterone, which are synthesized via a CYP17 C17,20-lyase

reaction from CYP17 substrate precursors such as pregnenolone (converted to DHEA by the CYP17 C17,20-lyase activity), and progesterone (converted to delta 4- androstenedione by the CYP17 C17,20-lyase activity). Progestagens such as progesterone are primarily synthesized in the corpus luteum. The androgens are responsible for, among other things, development of male secondary sex characteristics and are primarily synthesized in the testis. Other examples include the estrogens, which are also synthesized from a cholesterol precursor via a CYP17-mediated reaction. The estrogens are responsible for, among other things, the development of female secondary sex characteristics and they also participate in the ovarian cycle and are primarily synthesized in the ovary. Another group of CYP17 metabolites are the glucocorticoids, such as hydrocortisone (i.e. cortisol), which is synthesized from progesterone via a CYP17-mediated reaction. The glucocorticoids, among other functions, promote gluconeogenesis and the formation of glycogen and also enhance the degradation of fat. The glucocorticoids are primarily synthesized in the adrenal cortex.

The term "CYP17 metabolite" is further meant to include other steroid hormones which, although not necessarily synthesized by a CYP17-mediated reaction, may nonetheless be understood by the skilled artisan to be readily affected by an alteration in a CYP17-mediated activity. For example, the mineralocorticoids, such as aldosterone, are derived from cholesterol via a progesterone intermediate. Since progesterone is also converted to the glucocorticoids and sex steroids via CYP17-mediated reactions, an alteration of a CYP17 activity can alter the amount of progesterone available for conversion to aldosterone. For example, inhibition of CYP17 activity can increase the amount of progesterone available for conversion into aldosterone. Therefore, inhibition of CYP17 can lead to an increase in the level of aldosterone. The mineralocorticoids function, among other things, to increase reabsorption of sodium ions, chloride ions, and bicarbonate ions by the kidney, which leads to an increase in blood volume and blood pressure. The mineralocorticoids are primarily synthesized in the adrenal cortex.

The term "CYP17 metabolite-associated disease or disorder" refers to a disease or disorder which may be treated by alteration of the level of one or more CYP17 metabolites. Examples include a hormone dependent cancer, such as an androgen-dependent prostate cancer, which may be treated by inhibiting CYP17-mediated androgen synthesis, and an estrogen-dependent breast cancer or ovarian cancer, which may be treated by inhibiting CYP17-mediated estrogen synthesis. Other examples of "CYP17 metabolite-associated

diseases or disorders" are Cushing's disease, hypertension, prostatic hyperplasia, and glucocorticoid deficiency. Patients with Cushing's syndrome are relatively insensitive to glucocorticoid feedback and exhibit an oversecretion of cortisol devoid of a circadian cycle (see e.g. Newell-Price & Grossman (2001) *Ann. Endocrinol.* 62: 173-9). Another CYP17 metabolite-associated disease or disorder is hypertension. Mineralocorticoid excess causes hypertension by facilitating the sodium retention at renal tubules.

The term "derivative" of a compound refers to another compound which can be derived, e.g., by chemical synthesis, from the original compound. Thus a derivative of a compound has certain structural similarities with the original compound.

"Disease associated with an abnormal activity or level of a lyase" refers to diseases in which an abnormal activity or protein level of a lyase is present in certain cells, and in which the abnormal activity or protein level of the lyase is at least partly responsible for the disease.

A "disease associated with a lyase" refers to a disease that can be treated with a lyase inhibitor, such as the compounds disclosed herein.

A "lyase" refers to an enzyme having a lyase activity.

"Lyase activity" refers to the activity of an enzyme to catalyze the cleavage of the bond C17-C20 in 17α-hydroxy-pregnenolone and 17α-hydroxy-progesterone to form dehydroepiandrosterone (DHEA) and delta4-androstenedione, respectively. Lyase activity also refers to the cleavage of a similar bond in related compounds.

A "lyase inhibitor" is a compound which inhibits at least part of the activity of a lyase in a cell. The inhibition can be at least about 20%, preferably at least about 40%, even more preferably at least about 50%, 70%, 80%, 90%, 95%, and most preferably at least about 98% of the activity of the lyase.

A "patient" or "subject" to be treated by the subject method can mean either a human or non-human animal.

"Treating" a disease refers to preventing, curing or improving at least one symptom of a disease.

The following definitions pertain to the chemical structure of compounds:

The term "heteroatom" as used herein means an atom of nitrogen, oxygen, or sulfur.

The term "alkyl" refers to the radicals of saturated aliphatic groups, including straight-chain alkyl groups and branched-chain alkyl groups.

The term "cycloalkyl" (alicyclic) refers to radicals of cycloalkyl compounds, examples being cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, etc.

The term "aralkyl", as used herein, refers to an alkyl group substituted with an aryl group (e.g., an aromatic or heteroaromatic group).

The terms "alkenyl" and "alkynyl" refer to unsaturated aliphatic groups that contain at least one double or triple bond respectively.

Unless the number of carbons is otherwise specified, "lower alkyl" as used herein means an alkyl group but having from one to six carbons, preferably from one to four carbon atoms in its backbone structure. Likewise, "lower alkenyl" and "lower alkynyl" have similar chain lengths. Preferred alkyl groups are lower alkyls.

The term "aryl" as used herein means an aromatic group of 6 to 14 carbon atoms in the ring(s), for example, phenyl and naphthyl. As indicated, the term "aryl" includes polycyclic ring systems having two or more rings in which two or more carbons are common to two adjoining rings (the rings are "fused rings") wherein at least one of the rings is aromatic.

The term "heteroaryl" as used herein means an aromatic group which contains at least one heteroatom in at least one ring. Typical examples include 5-, 6- and 7-membered single-ring aromatic groups that may include from one to four heteroatoms. Examples include pyrrole, furan, thiophene, imidazole, oxazole, thiazole, triazole, tetrazole, pyrazole, pyridine, pyrazine, pyridazine and pyrimidine, and the like. These aryl groups may also be referred to as "aryl heterocycles" or "heteroaromatics."

The terms *ortho*, *meta* and *para* apply to 1,2-, 1,3- and 1,4-disubstituted benzenes, respectively. For example, the names 1,2-dimethylbenzene and *ortho*-dimethylbenzene are synonymous.

The terms "alkoxyl" or "alkoxy" as used herein refer to moiety in which an alkyl group is bonded to an oxygen atom, which is in turn bonded to the rest of the molecule. Examples are methoxy, ethoxy, propyloxy, *tert*-butoxy, etc.

As used herein, the term "nitro" means $-NO_2$; the term "halogen" designates -F, -Cl, -Br or -I; the term "sulfhydryl" means -SH; the term "hydroxyl" means -OH; and the term "sulfonyl" means $-SO_2-$.

The terms triflyl, tosyl, mesyl, and nonaflyl are art-recognized and refer to trifluoromethanesulfonyl, *p*-toluenesulfonyl, methanesulfonyl, and nonafluorobutanesulfonyl groups, respectively. The terms triflate, tosylate, mesylate, and nonaflate are art-recognized and refer to trifluoromethanesulfonate ester, *p*-toluenesulfonate ester, methanesulfonate ester, and nonafluorobutanesulfonate ester functional groups and molecules that contain said groups, respectively.

The abbreviations Me, Et, Ph, Tf, Nf, Ts, Ms represent methyl, ethyl, phenyl, trifluoromethanesulfonyl, nonafluorobutanesulfonyl, *p*-toluenesulfonyl and methanesulfonyl, respectively. A more comprehensive list of the abbreviations utilized by organic chemists of ordinary skill in the art appears in the first issue of each volume of the *Journal of Organic Chemistry*; this list is typically presented in a table entitled Standard List of Abbreviations. The abbreviations contained in said list are hereby incorporated by reference.

As used herein, the definition of each expression, e.g. alkyl, m, n, etc., when it occurs more than once in any structure, is intended to be independent of its definition elsewhere in the same structure.

It will be understood that "substitution" or "substituted with" includes the implicit proviso that such substitution is in accordance with permitted valence of the substituted atom and the substituent, and that the substitution results in a stable compound, e.g., which does not spontaneously undergo transformation such as by rearrangement, cyclization, elimination, etc.

As used herein, the term "substituted" is contemplated to include all permissible substituents of organic compounds. In a broad aspect, the permissible substituents include acyclic and cyclic, branched and unbranched, carbocyclic and heterocyclic, aromatic and nonaromatic substituents of organic compounds. Illustrative substituents include, for example, those described herein above. The permissible substituents can be one or more and the same or different for appropriate organic compounds. For purposes of this invention, the heteroatoms such as nitrogen may have hydrogen substituents and/or any permissible

substituents of organic compounds described herein which satisfy the valences of the heteroatoms.

The phrase "protecting group" as used herein means temporary substituents which protect a potentially reactive functional group from undesired chemical transformations. Examples of such protecting groups include esters of carboxylic acids, silyl ethers of alcohols, and acetals and ketals of aldehydes and ketones, respectively. The field of protecting group chemistry has been reviewed (Greene, T.W.; Wuts, P.G.M. *Protective Groups in Organic Synthesis*, 3rd ed.; Wiley: New York, 1999).

Abbreviations and Acronyms

When the following abbreviations are used throughout the disclosure, they have the following meaning:

amu	atomic mass units
Ar	argon
n-BuLi	butyllithium
CH_2Cl_2	methylene chloride
CPM	counts per minute
DMSO	dimethylsulfoxide
DMSO-d_6	dimethylsulfoxide-d_6
ES	electrospray (for mass spectrometry)
Et_3N	triethylamine
EtOAc	ethyl acetate
Et_2O	diethyl ether
EtOH	ethanol
g	gram
GCMS	gas chromatography/mass spectrometry
H_2	hydrogen gas
HCl	hydrochloric acid
^{1}H NMR	proton nuclear magnetic resonance
HPES	4-(2-Hydroxyethyl peperazine-1-ethane sulfonic acid)
HPLC	high performance liquid chromatography

KOH	potassium hydroxide
LC/MS	liquid chromatography / mass spectroscopy
M	molar
MeOH	methanol
mg	milligram
min	minute(s)
mL	milliliter
mm	millimeter
MS	mass spectrometry
m/z	mass to charge ratio (for mass spectrometry)
$NaHCO_3$	sodium bicarbonate
Na_2SO_4	sodium sulfate
NH_4Cl	ammonium chloride
OTf	trifluoroacetate (triflate)
OTs	*p*-toluenesulfonate (tosylate)
Pd/C	palladium on carbon
$POCl_3$	Phosphorous oxychloride
psi	pounds per square inch
R_f	TLC retention factor
rt	room temperature
SPA	Scintillation Proximity Assay
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TMS	tetramethylsilane
TLC	thin layer chromatography
t_R	retention time
µL	microliter
µM	micrometer
uv/vis	ultraviolet/visable

Compounds of the Invention

Exemplary compounds of the invention are set forth in Table 1 below. The compounds of Table 1 are producible from known compounds (or from starting materials which, in turn, are producible from known compounds), through the general preparative methods described in the General Methods or Examples.

Table 1. Exemplary Compounds of the Invention.

COMPOUND NAMES
2-benzyl-5-(3-pyridyl)tetrazole
2-[(3-methoxyphenyl)methyl]-5-(3-pyridyl)tetrazole
2-[(2-methylphenyl)methyl]-5-(3-pyridyl)tetrazole
2-[(3-methylphenyl)methyl]-5-(3-pyridyl)tetrazole
2-[(4-fluorophenyl)methyl]-5-(3-pyridyl)tetrazole
2-[(2-bromophenyl)methyl]-5-(3-pyridyl)tetrazole
2-[(3-bromophenyl)methyl]-5-(3-pyridyl)tetrazole
2-[(3-nitrophenyl)methyl]-5-(3-pyridyl)tetrazole
2-[(4-nitrophenyl)methyl]-5-(3-pyridyl)tetrazole
2-[(2-chlorophenyl)methyl]-5-(3-pyridyl)tetrazole
2-[(3-chlorophenyl)methyl]-5-(3-pyridyl)tetrazole
2-[(4-chlorophenyl)methyl]-5-(3-pyridyl)tetrazole
2-[(4-methylphenyl)methyl]-5-(3-pyridyl)tetrazole
2-[(3,4-dichlorophenyl)methyl]-5-(3-pyridyl)-1,2,3,4-tetrazole
2-[(2-nitrophenyl)methyl]-5-(3-pyridyl)-1,2,3,4-tetrazole
2-(2-naphthylmethyl)-5-(3-pyridyl)-1,2,3,4-tetrazole
2-(cyclohexylmethyl)-5-(3-pyridyl)-1,2,3,4-tetrazole
2-(cyclobutylmethyl)-5-(3-pyridyl)-1,2,3,4-tetrazole
2-(2-ethylbutyl)-5-(3-pyridyl)-1,2,3,4-tetrazole
4-{[5-(4-methyl-3-pyridyl)-1,2,3,4-tetraazol-2-yl]methyl}benzenecarbonitrile
5-(4-methyl(3-pyridyl))-2-[(4-nitrophenyl)methyl]-1,2,3,4-tetrazole
5-(4-methyl(3-pyridyl))-2-[(3-nitrophenyl)methyl]-1,2,3,4-tetrazole
2-[(4-chlorophenyl)methyl]-5-(4-methyl(3-pyridyl))-1,2,3,4-tetrazole
2-[(2-chlorophenyl)methyl]-5-(4-methyl(3-pyridyl))-1,2,3,4-tetrazole

COMPOUND NAMES
2-[(3,4-dichlorophenyl)methyl]-5-(4-methyl(3-pyridyl))-1,2,3,4-tetrazole
5-(4-methyl(3-pyridyl))-2-[(4-methylphenyl)methyl]-1,2,3,4-tetrazole
2-[(4-bromophenyl)methyl]-5-(4-methyl(3-pyridyl))-1,2,3,4-tetrazole
5-(4-methyl(3-pyridyl))-2-(2-phenylethyl)-1,2,3,4-tetrazole
2-(cyclohexylmethyl)-5-(4-methyl(3-pyridyl))-1,2,3,4-tetrazole
2-(2-ethylbutyl)-5-(4-methyl(3-pyridyl))-1,2,3,4-tetrazole

Certain compounds of the present invention may exist in particular geometric or stereoisomeric forms. The present invention contemplates all such compounds, including *cis*- and *trans*-isomers, *R*- and *S*-enantiomers, diastereomers, (D)-isomers, (L)-isomers, the racemic mixtures thereof, and other mixtures thereof, as falling within the scope of the invention. Additional asymmetric carbon atoms may be present in a substituent such as an alkyl group. All such isomers, as well as mixtures thereof, are intended to be included in this invention.

If, for instance, a particular enantiomer of a compound of the present invention is desired, it may be prepared by asymmetric synthesis, or by derivatizaton with a chiral auxiliary, where the resulting diastereomeric mixture is separated and the auxiliary group cleaved to provide the pure desired enantiomers. Alternatively, where the molecule contains a basic functional group, such as amino, or an acidic functional group, such as carboxyl, diastereomeric salts are formed with an appropriate optically-active acid or base, followed by resolution of the diastereomers thus formed by fractional crystallization or chromatographic means well known in the art, and subsequent recovery of the pure enantiomers.

Compounds may contain a basic functional group, such as amino or alkylamino, and are, thus, capable of forming pharmaceutically acceptable salts with pharmaceutically acceptable acids. The term "pharmaceutically acceptable salts" in this respect, refers to the relatively nontoxic, inorganic and organic acid addition salts of compounds of the present invention. These salts can be prepared *in situ* during the final isolation and purification of the compounds of the invention, or by separately reacting a purified compound of the invention in its free base form with a suitable organic or inorganic acid, and isolating the salt thus formed. Representative salts include the hydrobromide, hydrochloride, sulfate, bisulfate, phosphate, nitrate, acetate, valerate, oleate, palmitate, stearate, laurate, benzoate, lactate, phosphate, tosylate, citrate, maleate, fumarate, succinate, tartrate, napthylate,

mesylate, glucoheptonate, lactobionate, and laurylsulphonate salts and the like. (See, for example, Berge et al. (1977) "Pharmaceutical Salts", *J. Pharm. Sci.* 66:1-19).

Pharmaceutically acceptable salts of the subject compounds include the conventional nontoxic salts or quaternary ammonium salts of the compounds, e.g., from non-toxic organic or inorganic acids. For example, such conventional nontoxic salts include those derived from inorganic acids such as hydrochloric, hydrobromic, sulfuric, sulfamic, phosphoric, nitric, and the like; and the salts prepared from organic acids such as acetic, propionic, succinic, glycolic, stearic, lactic, malic, tartaric, citric, ascorbic, palmitic, maleic, hydroxymaleic, phenylacetic, glutamic, benzoic, salicyclic, sulfanilic, 2-acetoxybenzoic, fumaric, toluenesulfonic, methanesulfonic, ethane disulfonic, oxalic, isothionic, and the like.

In other cases, the compounds of the present invention may contain one or more acidic functional groups and, thus, are capable of forming pharmaceutically acceptable salts with pharmaceutically acceptable bases. These salts can be prepared *in situ* during the final isolation and purification of the compounds, or by separately reacting the purified compound in its free acid form with a suitable base, such as the hydroxide, carbonate or bicarbonate of a pharmaceutically acceptable metal cation, with ammonia, or with a pharmaceutically-acceptable organic primary, secondary or tertiary amine. Representative alkali or alkaline earth salts include the lithium, sodium, potassium, calcium, magnesium, and aluminum salts and the like. Representative organic amines useful for the formation of base addition salts include ethylamine, diethylamine, ethylenediamine, ethanolamine, diethanolamine, piperazine and the like. (See, for example, Berge et al., *supra*).

Contemplated equivalents of the compounds described above include compounds which otherwise correspond thereto, and which have the same general properties thereof (e.g., functioning as 17α-hydroxylase-C17,20-lyase inhibitors), wherein one or more simple variations of substituents are made which do not adversely affect the efficacy of the compound in binding to 17α-hydroxylase-C17,20-lyase receptors. In general, the compounds of the present invention may be prepared by the methods illustrated in the general reaction schemes as, for example, described below, or by modifications thereof, using readily available starting materials, reagents and conventional synthesis procedures. In these reactions, it is also possible to make use of variants which are in themselves known, but are not mentioned here.

Diseases that can be treated with the compounds of the invention

The present invention provides a method of inhibiting a lyase, e.g., 17α-hydroxylase-C17,20 lyase, comprising contacting a lyase with a compound of the invention. The activity

can be inhibited by at least 20%, preferably at least about 50%, more preferably at least about 60%, 70%, 80%, 90%, 95%, and most preferably at least about 98%. In one embodiment, the invention provides a method for inhibiting a lyase *in vitro*. In a preferred embodiment, the lyase is *in vivo* or *ex vivo*. For example, the invention provides methods for inhibiting a lyase in a cell, comprising contacting the cell with a compound of the invention, such that the activity of the lyase is inhibited. The cell may further be contacted with a composition stimulating the uptake of the compound into the cell, e.g., liposomes. In one embodiment, the invention provides a method for inhibiting a lyase in a cell of a subject, comprising administering to the subject a therapeutically effective amount of a compound of the present invention, or a formulation comprising a compound of the present invention, such that the lyase is inhibited in a cell of the subject. The subject can be one having a disease associated with a lyase, e.g., cancer. Preferred types of cancer that can be treated according to the invention include prostate cancer and breast cancer. Other diseases that can be treated include diseases in which it is desired to prevent or inhibit the formation of a hormone selected from the group consisting of the androgens testosterone and dihydrotestosterone (DHT) and the estrogens 17β-estradiol and estrone. Generally, any disease that can be treated by inhibiting the activity of a lyase, e.g., 17α-hydroxylase-C17,20-lyase, can be treated with the compounds of the invention.

In general, the invention provides methods and compositions for the treatment of CYP17 metabolite-associated diseases and disorders. Examples include particularly sex steroid hormone dependent cancers, such as androgen-dependent prostate cancer, which may be treated by inhibiting CYP17-mediated androgen synthesis, and estrogen-dependent breast cancer or ovarian cancer, which may be treated by inhibiting CYP17-mediated estrogen synthesis.

For example, adenocarcinoma of the prostate is a common disease that causes significant morbidity and mortality in the adult male population (see Han and Nelson (2000) Expert Opin. Pharmacother. 1: 443-9). Hormonal therapy for prostate cancer is considered when a patient fails with initial curative therapy, such as radical prostatectomy or definitive radiation therapy, or if he is found with an advanced disease. Hormonal agents have been developed to exploit the fact that prostate cancer growth is dependent on androgen. Non-steroidal anti-androgens (NSAAs) block androgen at the cellular level. Castration is another, albeit drastic means of decreasing androgens levels in order to treat or prevent prostate

cancer. The methods and compositions of the invention are useful in inhibiting the C17,20-lyase activity of CYP17 and thereby decreasing levels of androgen production and the associated growth of androgen-dependent cancers such as prostate cancer.

In another example, breast cancer, particularly breast cancer in postmenopausal women, can be treated by administration of a C17,20-lyase inhibitor of the invention because adrenal and ovarian androgens are the main precursors of the estrogens which stimulate the growth of hormone dependent breast cancer. In addition, breast cancer can be treated with inhibitors of aromatase that prevent interconversion of estrogens and adrenal and ovarian androgens (see Harris et al. (1983) Eur. J. Cancer Clin. Oncol. 19: 11). Patients failing to respond to aromatase inhibitors show elevated levels of androgens in response to aromatase inhibitor treatment (see Harris et al. (1988) Br. J. Cancer 58: 493-6). Accordingly sequential blockade to inhibit androgen production as well as inhibit aromatase may produce greater estrogen suppression and enhanced therapeutic effects in treating breast and other estrogen hormone-dependent forms of cancer. Therefore the inhibitors of the invention may be used alone or in combination with other drugs to treat or prevent hormone-dependent cancers such as breast and prostate cancer.

Furthermore, susceptibility to prostate cancer and breast cancer has been associated with particular polymorphic alleles of the CYP17 gene (see e.g. McKean-Cowdin (2001) Cancer Res. 61: 848-9; Haiman et al. (2001) Cancer Epidmeiol. Biomarkers 10: 743-8; Huang et al. (2001) Cancer Res. 59: 4870-5). Accordingly, the compositions of the invention are particularly suited to treating or preventing hormone-dependent cancers in individuals genetically predisposed to such cancers, particularly those predisposed due to an alteration in the CYP17 gene.

Another group of CYP17 metabolite-associated diseases or disorders amenable to treatment with the compositions and methods of the invention include those associated with mineralocorticoid excess such as hypertension caused by sodium retention at renal tubules. Such a mechanism operates in hypertension such as primary hyperaldosteronism and some forms of congenital adrenal hyperplasia. Recently, deficient cortisol metabolism in the aldosterone target organ has been recognized as a novel form of hypertension known as apparent mineralocorticoid excess. Disorders associated with mineralocorticoid synthesis include abnormalities of mineralocorticoid synthesis and/or metabolism which profoundly affect the regulation of electrolyte and water balance and of blood pressure (see e.g. Connell

et al. (2001) *Baillieres Best* Pract. Res. Clin. Endocrinol. Metab. 15:43-60). Characteristic changes in extracellular potassium, sodium and hydrogen ion concentrations are usually diagnostic of such disorders. Serious deficiency may be acquired, for example, in Addison's disease, or inherited. In most of the inherited syndromes, the precise molecular changes in specific steroidogenic enzymes have been identified. Mineralocorticoid excess may be caused by aldosterone or 11-deoxycorticosterone by inadequate conversion of cortisol to cortisone by 11β-hydroxysteroid dehydrogenase type 2 in target tissues, by glucocorticoid receptor deficiency or by constitutive activation of renal sodium channels. Changes in electrolyte balance and renin as well as the abnormal pattern of corticosteroid metabolism are usually diagnostic. Where these abnormalities are inherited (e.g. 11beta- or 17alpha-hydroxylase deficiencies, glucocorticoid remediable hyperaldosteronism (GRA), receptor defects, Liddle's syndrome), the molecular basis is again usually known and, in some cases, may provide the simplest diagnostic tests. Primary aldosteronism, although readily identifiable, presents problems of differential diagnosis, important because optimal treatment is different for each variant. Finally, a significant proportion of patients with essential hypertension show characteristics of mild mineralocorticoid excess, for example low renin levels. As described above, a decrease in CYP17 activity can result in an alteration in mineralorticoid (e.g. aldosterone) biosynthesis. Accordingly, the "CYP17 metabolite-associated diseases or disorders" of the invention would include those associated with altered levels of aldosterone production (e.g. hypertension, primary adrenal hyperplasia).

Still other examples of CYP17 metabolite-associated diseases or disorders" are Cushing's disease, prostatic hyperplasia, glucocorticoid deficiency, and endometrial cancer.

The subject that can be treated according to the invention can be a mammal, e.g., a primate, equine, canine, bovine, ovine, porcine, or feline. In preferred embodiments of this method, the mammal is a human. In other embodiments, the invention provides methods for inhibiting the lyase activity of enzymes that are present in organisms other than mammals, e.g., yeast and fungus, e.g., mildew. Certain compounds of the invention may function as antifungal compounds.

Methods of administering the compounds of the invention

The therapeutic methods of the invention generally comprise administering to a subject in need thereof, a pharmaceutically effective amount of a compound of the invention, or a salt, prodrug or composition thereof. The compounds of the invention can be

administered in an amount effective to inhibit the activity of a 17α-hydroxylase-C17,20-lyase. The compounds of this invention may be administered to mammals, preferably humans, either alone or, preferably, in combination with pharmaceutically acceptable carriers, excipients or diluents, in a pharmaceutical composition, according to standard pharmaceutical practice. The compounds can be administered orally or parenterally, including the intravenous, intramuscular, intraperitoneal, subcutaneous, rectal and topical routes of administration.

Toxicity and therapeutic efficacy of the compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD_{50}/ED_{50}. Compounds which exhibit large therapeutic indices are preferred. While compounds that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such reagents to the site of affected tissue in order to minimize potential damage to normal cells and, thereby, reduce side effects.

Data obtained from cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC_{50} (i.e., the concentration of the test compound which achieves a half-maximal inhibition of activity) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. The compounds of the invention have an IC_{50} less than 10 μM as determined by the biochemical or cellular assay described herein. Some compounds of the invention are effective at concentrations of 10 nM, 100 nM, or 1 μM. Based on these numbers, it is possible to derive an appropriate dosage for administration to subjects.

Formation of prodrugs is well known in the art in order to enhance the properties of the parent compound. Such properties include solubility, absorption, biostability and release

time (see *"Pharmaceutical Dosage Form and Drug Delivery Systems"* (Sixth Edition), edited by Ansel *et al.*, publ. by Williams & Wilkins, pgs. 27-29, (1995)). Commonly used prodrugs of the disclosed compounds can be designed to take advantage of the major drug biotransformation reactions and are also to be considered within the scope of the invention. Major drug biotransformation reactions include *N*-dealkylation, O-dealkylation, aliphatic hydroxylation, aromatic hydroxylation, *N*-oxidation, S-oxidation, deamination, hydrolysis reactions, glucuronidation, sulfation and acetylation (see *Goodman and Gilman's The Pharmacological Basis of Therapeutics* (Ninth Edition), editor Molinoff *et al.*, publ. by McGraw-Hill, pages 11-13, (1996)).

The pharmaceutical compositions can be prepared so that they may be administered orally, dermally, parenterally, nasally, ophthalmically, otically, sublingually, rectally or vaginally. Dermal administration includes topical application or transdermal administration. Parenteral administration includes intravenous, intraarticular, intramuscular, intraperitoneal, and subcutaneous injections, as well as use of infusion techniques. One or more compounds of the invention may be present in association with one or more non-toxic pharmaceutically acceptable ingredients and optionally, other active anti-proliferative agents, to form the pharmaceutical composition. These compositions can be prepared by applying known techniques in the art such as those taught in *Remington's Pharmaceutical Sciences* (Fourteenth Edition), Managing Editor, John E. Hoover, Mack Publishing Co., (1970) or *Pharmaceutical Dosage Form and Drug Delivery Systems* (Sixth Edition), edited by Ansel *et al.*, publ. by Williams & Wilkins, (1995).

As indicated above, pharmaceutical compositions containing a compound of the invention may be in a form suitable for oral use, for example, as tablets, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules, emulsions, hard or soft capsules, or syrups or elixirs. Compositions intended for oral use may be prepared according to any method known to the art for the manufacture of pharmaceutical compositions and such compositions may contain one or more agents selected from the group consisting of sweetening agents, flavoring agents, coloring agents and preserving agents in order to provide pharmaceutically acceptable preparations. Tablets contain the active ingredient in admixture with non-toxic pharmaceutically acceptable excipients which are suitable for the manufacture of tablets. These excipients may be, for example, inert diluents, such as calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents, for example, microcrystalline cellulose,

sodium crosscarmellose, corn starch, or alginic acid; binding agents, for example starch, gelatin, polyvinyl-pyrrolidone or acacia; and lubricating agents, for example, magnesium stearate, stearic acid or talc. The tablets may be uncoated or they may be coated by known techniques to mask the unpleasant taste of the drug or delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a water soluble taste masking material such as hydroxypropylmethyl-cellulose or hydroxypropylcellulose, or a time delay material such as ethyl cellulose, cellulose acetate buryrate may be employed.

Formulations for oral use may also be presented as hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin, or as soft gelatin capsules wherein the active ingredient is mixed with water soluble carrier such as polyethyleneglycol or an oil medium, for example peanut oil, liquid paraffin, or olive oil.

Aqueous suspensions contain the active material in admixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients are suspending agents, for example sodium carboxymethylcellulose, methylcellulose, hydroxypropylmethyl-cellulose, sodium alginate, polyvinyl-pyrrolidone, gum tragacanth and gum acacia; dispersing or wetting agents may be a naturally occurring phosphatide, for example lecithin; or condensation products of an alkylene oxide with fatty acids, for example polyoxyethylene stearate; or condensation products of ethylene oxide with long chain aliphatic alcohols, for example heptadecaethylene-oxycetanol; or condensation products of ethylene oxide with partial esters derived from fatty acids and a hexitol such as polyoxyethylene sorbitol monooleate; or condensation products of ethylene oxide with partial esters derived from fatty acids and hexitol anhydrides, for example polyethylene sorbitan monooleate. The aqueous suspensions may also contain one or more preservatives, for example ethyl or n-propyl p-hydroxybenzoate, one or more coloring agents, one or more flavoring agents, and one or more sweetening agents, such as sucrose, saccharin or aspartame.

Oily suspensions may be formulated by suspending the active ingredient in a vegetable oil, for example arachis oil, olive oil, sesame oil or coconut oil, or in mineral oil such as liquid paraffin. The oily suspensions may contain a thickening agent, for example beeswax, hard paraffin or cetyl alcohol. Sweetening agents such as those set forth above, and flavoring agents may be added to provide a palatable oral preparation. These

compositions may be preserved by the addition of an anti-oxidant such as butylated hydroxyanisol or alpha-tocopherol.

Dispersible powders and granules suitable for preparation of an aqueous suspension by the addition of water provide the compound of the invention in admixture with a dispersing or wetting agent, suspending agent and one or more preservatives. Suitable dispersing or wetting agents and suspending agents are exemplified by those already mentioned above. Additional excipients, for example sweetening, flavoring and coloring agents, may also be present. These compositions may be preserved by the addition of an anti-oxidant such as ascorbic acid.

Pharmaceutical compositions of the invention may also be in the form of an oil-in-water emulsions. The oily phase may be a vegetable oil, for example olive oil or arachis oil, or a mineral oil, for example liquid paraffin or mixtures of these. Suitable emulsifying agents may be naturally occurring phosphatides, for example soy bean lecithin, and esters or partial esters derived from fatty acids and hexitol anhydrides, for example sorbitan monooleate, and condensation products of the said partial esters with ethylene oxide, for example polyoxyethylene sorbitan monooleate. The emulsions may also contain sweetening, flavouring agents, preservatives and antioxidants.

Syrups and elixirs may be formulated with sweetening agents, for example glycerol, propylene glycol, sorbitol or sucrose. Such formulations may also contain a demulcent, a preservative, flavoring and coloring agents and antioxidant.

Pharmaceutical compositions may be in the form of a sterile injectable aqueous solutions. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution.

Sterile injectable preparation may also be a sterile injectable oil-in-water microemulsion where the compound of the invention is dissolved in the oily phase. For example, the active ingredient may be first dissolved in a mixture of soybean oil and lecithin. The oil solution is then introduced into a water and glycerol mixture and processed to form a microemulation.

The injectable solutions or microemulsions may be introduced into a patient's blood stream by local bolus injection. Alternatively, it may be advantageous to administer the solution or microemulsion in such a way as to maintain a constant circulating concentration

of the active compound. In order to maintain such a constant concentration, a continuous intravenous delivery device may be utilized. An example of such a device is the Deltec CADD-PLUS™ model 5400 intravenous pump.

The pharmaceutical compositions may be in the form of a sterile injectable aqueous or oleagenous suspension for intramuscular and subcutaneous administration. This suspension may be formulated according to the known art using those suitable dispersing or wetting agents and suspending agents which have been mentioned above. The sterile injectable preparation may also be a sterile injectable solution or suspension in a nontoxic parenterally acceptable diluent or solvent, for example as a solution in 1,3-butane diol. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

Compounds of the invention may also be administered in the form of a suppository for rectal administration of the drug. These compositions can be prepared by mixing the drug with a suitable non-irritating excipient which is solid at ordinary temperatures but liquid at the rectal temperature and will therefore melt in the rectum to release the drug. Such materials include cocoa butter, glycerinated gelatin, hydrogenated vegetable oils, mixtures of polyethylene glycols of various molecular weights and fatty acid esters of polyethylene glycol.

For topical use, creams, ointments, jellies, solutions or suspensions, etc., containing the compound of the invention can be employed. For purposes of this application, topical application shall include mouth washes and gargles.

The compounds for the present invention can be administered in intranasal form via topical use of suitable intranasal vehicles and delivery devices, or via transdermal routes, using those forms of transdermal skin patches well known to those of ordinary skill in the art. To be administered in the form of a transdermal delivery system, the dosage administration will preferably be continuous rather than intermittent throughout the dosage regimen.

The compounds of the invention may also be co-administered with other well known therapeutic agents that are selected for their particular usefulness against the condition that is

being treated. The compounds may be administered simultaneously or sequentially. For example, the active compounds may be useful in combination with known anti-cancer and cytotoxic agents. Similarly, the active compounds may be useful in combination with agents that are effective in the treatment and prevention of osteoporosis, inflammation, neurofibromatosis, restinosis, and viral infections. The active compounds may also be useful in combination with inhibitors of other components of signaling pathways of cell surface growth factor receptors.

Drugs that can be co-administered to a subject being treated with a compound of the invention include antineoplastic agents selected from vinca alkaloids, epipodophyllotoxins, anthracycline antibiotics, actinomycin D, plicamycin, puromycin, gramicidin D, taxol, colchicine, cytochalasin B, emetine, maytansine, or amsacrine. Methods for the safe and effective administration of most of these chemotherapeutic agents are known to those skilled in the art. In addition, their administration is described in the standard literature. For example, the administration of many of the chemotherapeutic agents is described in the "Physicians' Desk Reference" (PDR), 1996 edition (Medical Economics Company, Montvale, N.J. 07645-1742, USA).

Radiation therapy, including x-rays or gamma rays which are delivered from either an externally applied beam or by implantation of tiny radioactive sources, may also be used in combination with a compound of the invention to treat a disease, e.g., cancer.

When a composition according to this invention is administered into a human subject, the daily dosage will normally be determined by the prescribing physician with the dosage generally varying according to the age, weight, and response of the individual patient, as well as the severity of the patient's symptoms.

Kits of the invention

In one embodiment, a compound of the invention, materials and/or reagents required for administering the compounds of the invention may be assembled together in a kit. When the components of the kit are provided in one or more liquid solutions, the liquid solution preferably is an aqueous solution, with a sterile aqueous solution being particularly preferred.

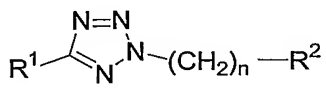
The kit may further comprise one or more other drugs, e.g., a chemo- or radiotherapeutic agent. These normally will be a separate formulation, but may be formulated into a single pharmaceutically acceptable composition. The container means may itself be geared for administration, such as an inhalant, syringe, pipette, eye dropper, or other such like apparatus, from which the formulation may be applied to an infected area of the body, such as the lungs, or injected into an animal, or even applied to and mixed with the other components of the kit.

The compositions of these kits also may be provided in dried or lyophilized forms. When reagents or components are provided as a dried form, reconstitution generally is by the addition of a suitable solvent. It is envisioned that the solvent also may be provided in another container means. The kits of the invention may also include an instruction sheet defining administration of the agent. Kits may also comprise a compound of the invention, labeled for detecting lyases.

The kits of the present invention also will typically include a means for containing the vials in close confinement for commercial sale such as, e.g., injection or blow-molded plastic containers into which the desired vials are retained. Irrespective of the number or type of containers, the kits of the invention also may comprise, or be packaged with a separate instrument for assisting with the injection/administration or placement of the ultimate complex composition within the body of an animal. Such an instrument may be an inhalant, syringe, pipette, forceps, measured spoon, eye dropper or any such medically approved delivery vehicle. Other instrumentation includes devices that permit the reading or monitoring of reactions or amounts of compounds or polypeptides.

General Method for the Preparation of Compounds of Formula I.

3-Pyridyl tetrazoles of Formula I, wherein R^1, R^2 and n are as described in claim 1



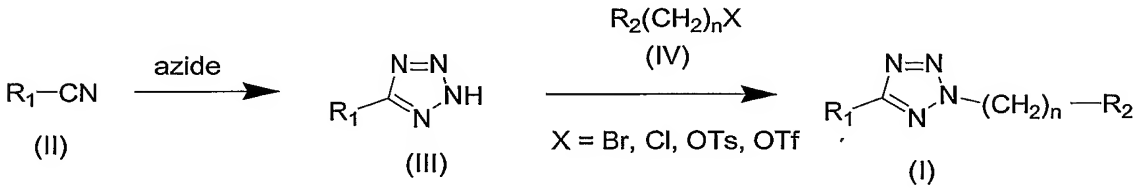
Formula I

are prepared by the general method described below, according to methods described below, or according to methods commonly employed in the art. The 3-cyanopyridines II used to prepare compounds of Formula I are commercially available, are prepared according to

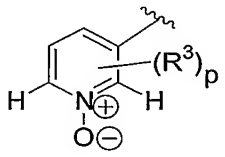
methods described below to prepare Intermediates A-F or are prepared according methods described in the following references: Comins, D. L., Smith, R., Stroud, E., Heterocycles, Vol. 22, No. 2, 1984, 339; Leete, E.; Leete, S. A. S., J. Org. Chem. Vol. 43, No. 11, 1978, 2122; Kim, J. G.; Yu, D. S.; Moon, S. H.; Park, J.; Park, W. W. J. Korean Chem. Soc. Vol. 37, No. 9, 1993, 826. Other methods commonly employed in the art may also be used to prepare 3-cyanopyridines II.

Treatment of II with sodium azide and ammonium chloride in a polar solvent such as DMF, DMA, DMSO, N-methylpyrrolidinone, or water at a temperature of about 80 – 180 °C provides 5-(3-pyridyl)tetrazoles III. Preferably the solvent is DMF and the temperature is about 120-140 °C. Other methods commonly employed in the art to prepare aryl tetrazoles from aryl nitriles may be used to prepare 5-(3-pyridyl)tetrazoles III; representative references are: Musicki, B.; Verert, J.-P. Tetrahedron Lett. Vol 35, No 50, 1994, 9391; Wittenberger, S. J.; Donner, B. G., J. Org, Chem., Vol 58, No. 15, 1993, 4139; Kim, K. S.; Qian, L; Bird, J. E.; Dickinson, K. E. J.; Moreland, S.; Shaeffer, T. R.; Waldron, T. L.; Delaney, C. L.; Weller, H. N.; Miller, A. V., J. Med. Chem., Vol. 36, No. 16, 1993, 2335.

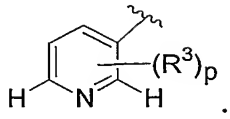
Tetrazoles III are treated with alkylating agent IV using General Methods A or B to prepare 3-(pyridyl)tetrazoles of Formula I. Alkylating agents IV are commercially available or are prepared according to methods commonly employed in the art to prepare benzyl/phenethyl bromides, chlorides, tosylates and triflates.



A Formula I compound where R^1 represents



may be prepared by standard N-oxidation conditions from the corresponding Formula I compound where R^1 represents



. An example of such conditions are hydrogen peroxide in acetic acid at a temperature of about 80 °C for about four hours.

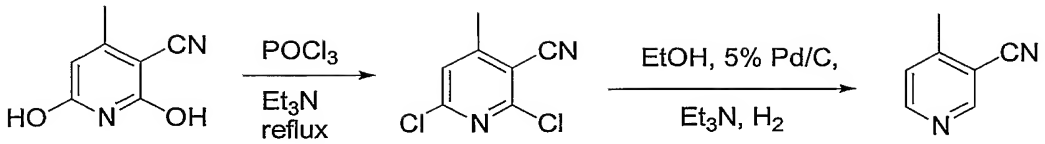
The invention is further illustrated by the following examples which should not be construed as limiting in any way. The contents of all cited references (including literature references, issued patents, published patent applications as cited throughout this application) are hereby expressly incorporated by reference.

Examples

Preparation of the compounds of the invention

General. All reagents are commercially available unless otherwise specified. Reagents were used as received unless otherwise specified. Proton NMR data is reported downfield from TMS; coupling constants are in hertz. LC/MS mass spectral data were obtained using a Hewlett-Packard 1100 HPLC equipped with a quaternary pump, a variable wavelength detector set at 254 nm, a YMC pro C-18 column (2 x 23 mm, 120A), and a Finnigan LCQ ion trap mass spectrometer with electrospray ionization. Spectra were scanned from 120-1200 amu using a variable ion time according to the number of ions in the source. The eluents were A: 2% acetonitrile in water with 0.02% TFA and B: 2% water in acetonitrile with 0.018% TFA. Gradient elution from 10% B to 95% B over 3.5 minutes at a flowrate of 1.0 mL/min was used with an initial hold of 0.5 minutes and a final hold at 95% B of 0.5 minutes. Total run time was 6.5 minutes. Purification by HPLC was performed using a Gilson HPLC system (UV/VIS-155 detector, 215 liquid handler, 306 pumps, 819 injection valve and an 811C mixer, the column was a YMC Pro C18 (75 x 30, 5μm, 120A); the eluents were A: water with 0.1% TFA, and B: acetonitrile with 0.1% TFA; gradient elution from 10% B to 90% B over 12 minutes with a final hold at 90% B for 2 minutes; flowrate was 25 mL per minute. NMR data are in agreement with the structures of all prepared compounds. Elemental analyses were obtained at Robertson Microlit Laboratories, Madison NJ. Melting points are uncorrected.

Preparation of Intermediate A: 4-Methyl-3-cyanopyridine.

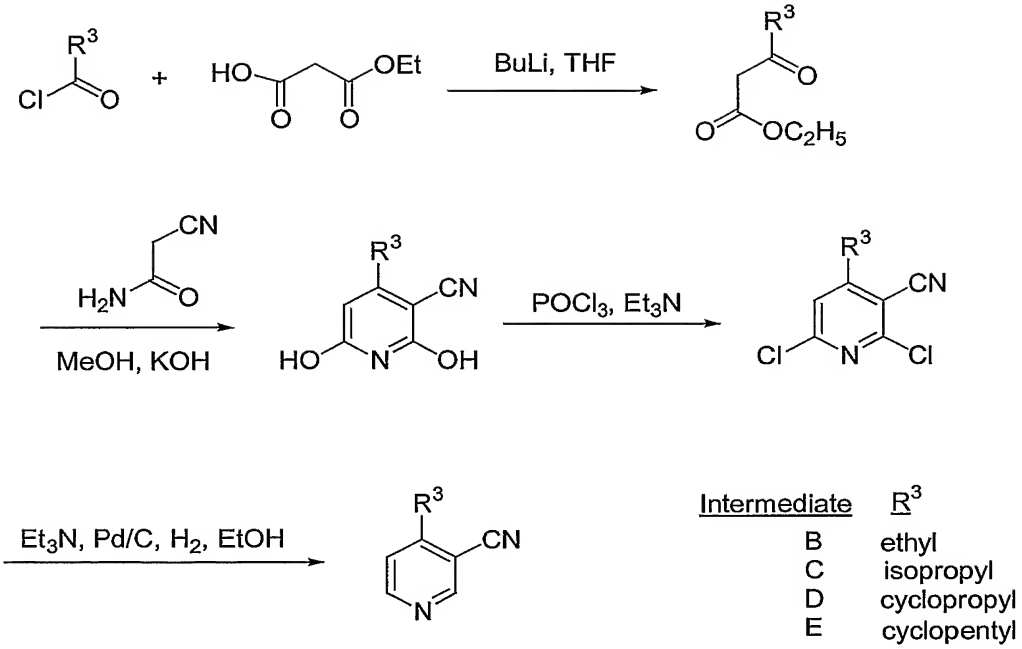


(A)

Step 1. 2,6-Dihydroxy-4-methyl-3-pyridinecarbonitrile (150 g, 1 mol) and phosphorus oxychloride (600 mL, 6.4 mol) were stirred under an argon atmosphere and triethylamine (300 mL, 2.1 mol) was added. After refluxing for 16 hours, the mixture was concentrated *in vacuo*, and the residue was partitioned between ice water (6 L) and dichloromethane (2 L). The organic phase was washed with aqueous sodium bicarbonate solution, then with brine, dried (Na_2SO_4), and then filtered through a pad of silica gel (465 g) on a sintered glass funnel. Elution with dichloromethane and concentration of the filtrate *in vacuo* afforded 109.6 g (58.6%) of 2,6-dichloro-4-methyl-3-cyanopyridine as a colorless crystalline solid, mp 108-110 °C: TLC R*f* 0.23 (1:1 hexane-dichloromethane, R*f* 0.31 (3:1 hexanes-EtOAc); ^{1}H NMR ($CDCl_3$) δ 7.3 (d, 1H), 2.3 (s, 3H); GCMS *m/z* 187 ($M+H^+$).

Step 2. 2,6-Dichloro-4-methyl-3-cyanopyridine (40.8 g, 0.22 mol) was dissolved in anhydrous ethanol (680 mL) and triethylamine (120 mL) by warming, and the solution hydrogenated over 5% palladium on carbon at 10 psi of hydrogen. Upon completion of the reaction, catalyst was removed by filtration. The filtrate was concentrated *in vacuo*. The resulting solid was triturated with ether, filtered, and then concentrated *in vacuo* to afford 16.3 mg (63.1%) of 4-methyl-3-cyanopyridine as colorless needles: mp, slowly melts 40-45°C; ^{1}H NMR ($CDCl_3$) δ 8.8 (s, 1H), 8.5 (d, 1H, J = 5), 7.3 (d, 1 H, J = 5), 2.6 (s, 3H). GCMS *m/z* 118 (M+).

General Method for the Preparation of 4-Substituted-3-Cyanopyridines: Preparation of 4-Ethyl-3-Cyanopyridine (Intermediate B), 4-(2-Propyl)-3-cyanopyridine (Intermediate C), 4-Cyclopropyl-3-cyanopyridine (Intermediate D), and 4-Cyclopentyl-3-cyanopyridine (Intermediate E)



Intermediate	R^3
B	ethyl
C	isopropyl
D	cyclopropyl
E	cyclopentyl

Step 1. *mono*-Ethyl malonate (35.0 g, 265 mmol) and THF (300 mL) is placed in a 500 mL round-bottomed flask and cooled to –70 °C under Ar. To this solution is added 330 mL of 1.6 M *n*-BuLi (2.0 equiv., 530 mmol) slowly and the solution allowed to stir for 10 min at –70 °C. The acid chloride, where R^3 is as defined above, is added to the solution slowly, stirred for one more hour at –70 °C, and then the reaction temperature is allowed to go to rt overnight. The solution is concentrated *in vacuo* and the residue is partitioned between HCl solution (1N, 200 mL) and Et_2O (2 x 300 mL). The organic layer is washed sequentially with $NaHCO_3$ (saturated 200 mL) and H_2O (200 mL), then dried over Na_2SO_4. The filtrate is concentrated and the crude product is purified by chromatography using hexanes-EtOAc (95:5). The average yield of the beta-ketoester is generally about 30-50%.

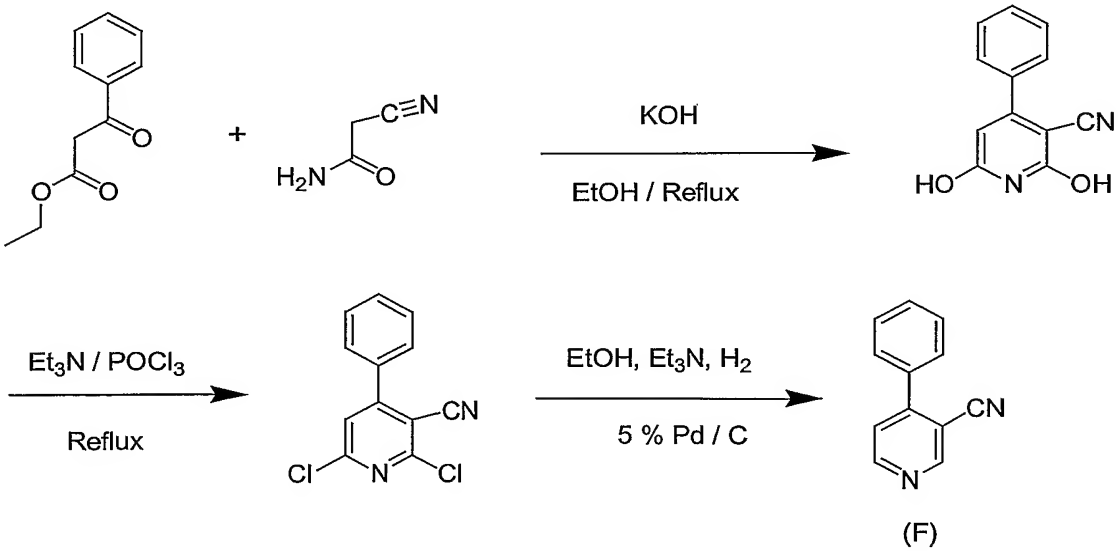
Step 2. The beta-ketoester (347 mmol) and 2-cyanoacetamide (347 mmol) are placed in a 500 mL round-bottomed flask and dissolved in 100 mL of THF under Ar. To this solution is slowly added a solution of KOH (1.1 equiv., 25.2 g, 382 mmol) in 150 mL MeOH. The solution allowed to stir at 70 °C for 8 hours, during which time a solid slowly forms. The reaction mixture is cooled to rt and the solid is filtered. The solid is dissolved in warm water (250 mL) and concentrated. HCl is added slowly until the pH is between 1 and 2. The resulting solid is filtered and dried to afford the 4-substituted-2,6-dihydroxy-3-cyanopyridine. The average yields of the 4-substituted-2,6-dihydroxy-3-cyanopyridines are

generally about 30-90%.

Step 3. In a 500 mL round-bottomed flask are placed the 4-substituted-2,6-dihydroxy-3-cyanopyridine (314 mmol) and $POCl_3$ (3.3 equiv, 1035 mmol) under Ar. Triethylamine (471 mmol, 65.5 mL) is added very slowly using an ice bath for cooling. The reaction mixture is heated to 130 °C for 8 hours under Ar after the addition finished. After cooling to rt, the reaction mixture is concentrated *in vacuo* and poured into ice (150 g). The residue is partitioned between CH_2Cl_2 (3 x 200 mL) and ice water. The separated organic layer is washed sequentially with $NaHCO_3$ (saturated 200 mL) and H_2O (200 mL), and dried over Na_2SO_4. The filtrate is concentrated and purified by chromatography using hexanes-EtOAc (80:20) as eluant. The average yield of the 4-substituted-2,6-dichloro-3-cyanopyridines is generally about 35-50%.

Step 4. In a 500 mL round-bottomed flask are placed the 4-substituted-2,6-dichloro-3-cyanopyridines (232 mmol), 10% Pd/C (2.0 g), Et_3N (927 mmol, 130 mL) and EtOH (300 mL). The mixture is hydrogenated at atmospheric pressure for 24 to 48 hours at rt. The catalyst is removed by filtration and the filtrate is concentrated. The residue is partitioned between CH_2Cl_2 (3 x 200 mL) and H_2O (200 mL), and then the separated organic layer is dried over Na_2SO_4. Concentration and purification by chromatography using hexanes-EtOAc (95:5) affords the 4-substituted-3-cyanopyridines in an average yields of about 85-95%.

Preparation of Intermediate F: 4-Phenyl-3-cyanopyridine



(F)

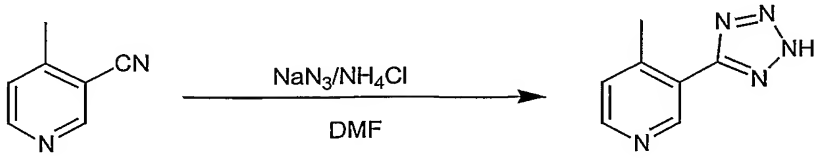
Step1. Ethyl 3-oxo-3-phenylpropanoate (51.9 mL, 0.300 mol) and 2-cyanoacetamide (25.2 g, 0.300 mol) were charged into ethanol (100 mL). The mixture was heated to 50 °C under

argon. To this reaction was charged potassium hydroxide (21.8 g, 0.330 mol) in 100 mL of ethanol via an additional funnel. The reaction was refluxed for approximately 17 hours, cooled to room temperature, and then filtered. The solid was washed with ethanol and then dried *in vacuo* overnight at 45 °C. This yielded 2,6-dihydroxy-4-phenylnicotinonitrile as a white solid, 12.5 g (19.6 %).

Step 2. 2,6-Dihydroxy-4-phenylnicotinonitrile (6.0 g, 28.2 mol) and triethylamine (4.2 mL, 0.0306 mol) were charged together into a round-bottomed flask. To this via syringe was added phosphorus oxychloride (8.2 mL, 90.4 mol). The reaction mixture was then refluxed for 17 hours under argon. It was then concentrated to an oil under reduced pressure to remove excess $POCl_3$. The oil was then poured slowly into a beaker with ice-water. The brown precipitate formed that was filtered, washed with copious amounts of water, and then dried *in vacuo* overnight at 45 °C. Purification by silica gel chromatography (mobile phase dichloromethane yielded 3.83 g (54.5%) of 2,6-dichloro-4-phenylnicotinonitrile as an off-white solid.

Step 3. A dry round-bottomed flask was charged with 5 % palladium on carbon (0.38 g, cat.) and anhydrous ethanol (5 mL). In another flask was charged 2,6-dichloro-4-phenylnicotinonitrile (3.83 g, 0.0154 mol), triethylamine (8.57 mL, 0.0615 mol) and more ethanol (80 mL, anhydrous). This was transferred to the reaction flask and this flask was then purged with argon. The flask was evacuated and again purged with argon; this was repeated twice more. Finally, a balloon containing hydrogen gas was attached to the flask and the reaction was then purged with hydrogen, then evacuated. Then hydrogen was released into the reaction flask and the mixture was hydrogenated for 48 hours. The reaction mixture was filtered and washed with ethanol. The filtrate was concentrated and the resulting oil was purified by column chromatography (mobile phase 20% EtOAc/hexane). 4-Phenylnicotinonitrile was obtained as a white solid was obtained in a 2.0 g (72%) yield.

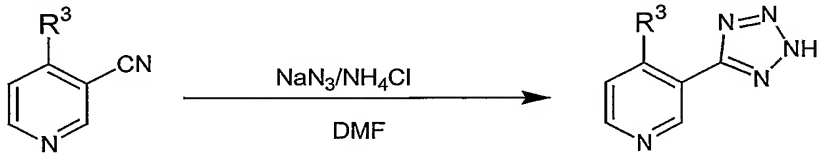
Preparation of Intermediate G: 5-(4-Methyl-3-pyridinyl)-2H-tetrazole.



(G)

To a solution of 4-methyl-3-cyanopyridine (5.0 g, 42.3 mmol) in DMF (42 mL) at room temperature were added sodium azide (3.29 g, 50.6 mmol) and ammonium chloride (2.71 g, 50.7 mmol). The resulting suspension was then heated to 130 °C with stirring. The reaction mixture was heated for 2 days. After it was cooled to rt, the suspension was filtered and the filtrate was concentrated to an oil. Upon standing the oil solidified. Ice water (15-20 mL) was added and the suspension was stirred for 15 min. A solid was collected by filtration, washed with small amount of water and dried under vacuum to give 2.31 g (33.9%) of the title compound as a colorless solid, mp 219.8 -221.4 °C. The combined filtrates were acidified with 2 N HCl to pH 5 to 6. The solution was concentrated to dryness. A small amount of water was added to the residue solid. The mixture was stirred, filtered, and then the solid was dried under vacuum to give an additional 1.05 g of product. This procedure was repeated to the filtrate to give a third batch of product (0.76 g). The overall yield was 60.4%: ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.85 (s, 1H), 8.58 (d, 1H), 7.47 (d, 1H), 2.52 (s, 3H); MS (ES) *m/z* 162 $(M+H)^+$. Anal. Calcd for $C_7H_7N_5$: C, 52.17; H, 4.38; N, 43.45. Found: C, 52.28; H, 4.28; N, 43.39.

General Method for the Preparation of 5-(4-Substituted-3-pyridinyl)-2*H*-tetrazoles: 5-(4-Ethyl-3-pyridinyl)-2*H*-tetrazole (Intermediate H), 5-(4-(2-Propyl)-3-pyridinyl)-2H-tetrazole (Intermediate I), 5-(4-Cyclopropyl-3-pyridinyl)-2*H*-tetrazole (Intermediate J), 5-(4-Cyclopentyl-3-pyridinyl)-2*H*-tetrazole (Intermediate K), and 5-(4-Phenyl-3-pyridinyl)-2*H*-tetrazole (Intermediate L).

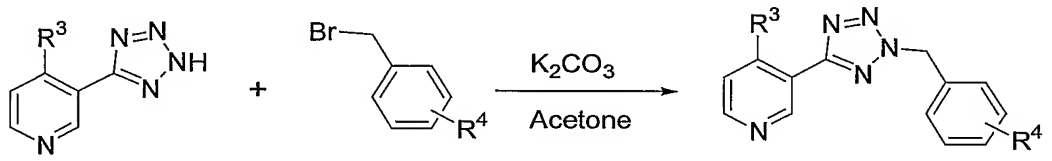


Intermediate	R^3
H	Et
I	*i*-Pr
J	*cyc*-Pr
K	*cyc*-Pent
L	Ph

Sodium Azide (50 mmol) and ammonium chloride (50.1 mmol) are sequentially added to a solution of 4-substituted-3-cyanopyridine (41.7 mmol) in DMF (40 mL) at room

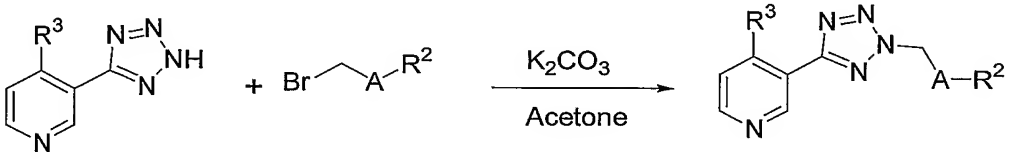
temperature and the resulting suspension is then heated to 130 °C with stirring. The reaction mixture is heated for 2-3 days. After cooling to rt, the suspension is filtered and the filtrate concentrated. Ice water (15-20 mL) is added and the mixture stirred for 15 min. The solid is collected by filtration, washed with small amount of water and dried under vacuum to give Intermediates H-L. A second crop can normally be obtained by acidifying the combined filtrates with 2 N HCl to pH 5 to 6, concentrating to dryness, triturating the residue with water, and then filtering and drying. The average yield of Intermediates H-L is 20-50%. The products are confirmed NMR and MS.

General Method A.



A suspension of 5-(3-pyridyl)-1*H*-tetrazole (0.68 mmol), potassium carbonate (0.74 mmol) and the substituted benzylbromide (0.74 mmol) in reagent grade acetone (10 mL) is refluxed overnight. The reaction is cooled and the solvent evaporated *in vacuo*. The residue is triturated in CH_2Cl_2 and filtered. Solvent from the filtrate is evaporated *in vacuo* and then purified via column chromatography (10-20% EtOAc/hexane) to afford the target alkylated tetrazoles in 70-90% yield. The products are confirmed by NMR and MS.

General Method B.

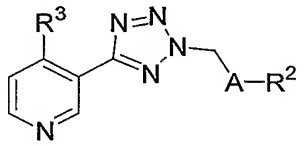


A mixture of the 5-(4-(R^1)-3-pyridyl)-1H-tetrazole (90-100 mg), potassium carbonate (1.2 equiv) and the substituted alkyl or arylbromide (1.2 equiv) in reagent grade acetone (10 mL) is refluxed overnight. The reaction is cooled and the solvent evaporated *in vacuo*. Water (5 mL) and dichloromethane (5 mL) is added and the mixture is agitated. The dichloromethane

layer is removed and the aqueous layer is extracted again with dichloromethane (5 mL). The combined dichloromethane layer is concentrated and purified by HPLC. Pooling pure fractions and concentration afford the target alkylated tetrazoles. The products are confirmed by NMR and MS.

5

Table 1. Examples 1 – 30 : Tetrazole Derivatives Prepared from 5-(3-pyridyl)-1H-tetrazole or 5-(4-Methyl-3-pyridyl)-1H-tetrazole by General Methods A and B.



Ex. No.	R^3	A	R^2	t_R min [a,b]	MS[a,b] $(M+H)^+$	TLC Rf (solvent)	General Method
1	H	Phenyl	H	--	238	Rf= 0.32 (25% EtOAc/hexanes)	A
2	H	Phenyl	3-OMe	--	268	Rf= 0.21 (25% EtOAc/hexanes)	A
3	H	Phenyl	2-Me	--	252	Rf= 0.27 (25% EtOAc/hexanes)	A
4	H	Phenyl	3-Me	--	252	Rf= 0.38 (25% EtOAc/hexanes)	A
5	H	Phenyl	4-F	--	256	Rf= 0.29 (25% EtOAc/hexanes)	A
6	H	Phenyl	2-Br	--	316 (M+)	Rf= 0.329 (25% EtOAc/hexanes)	A
7	H	Phenyl	3-Br	--	316 (M+)	Rf= 0.27 (25% EtOAc/hexanes)	A
8	H	Phenyl	3-NO_2	--	283	Rf= 0.36 (25% EtOAc/hexanes)	A
9	H	Phenyl	4-NO_2	--	283	Rf= 0.36 (25% EtOAc/hexanes)	A
10	H	Phenyl	2-Cl	--	272	Rf= 0.27 (25%	A

Ex. No.	R^3	A	R^2	t_R min [a,b]	MS[a,b] $(M+H)^+$	TLC Rf (solvent)	General Method
						EtOAc/hexanes)	
11	H	Phenyl	3-Cl	--	272	Rf= 0.26 (25% EtOAc/hexanes)	A
12	H	Phenyl	4-Cl	2.48	272	Rf= 0.15 (30% EtOAc/hexanes)	A
13	H	Phenyl	4-Me	--	252	Rf= 0.43 (25% EtOAc/hexanes)	A
14	H	Phenyl	3,4-diCl	2.73	305.99	Rf= 0.15 (30% EtOAc/hexanes)	B
15	H	Phenyl	2-NO_2	2.53	283	Rf= 0.08 (30% EtOAc/hexanes)	B
16	H	2-Naphthyl	H	2.68	288.07	Rf= 0.17 (30% EtOAc/hexanes)	B
17	H	Cyclohexyl	H	2.61	244.13	Rf= 0.29 (30% EtOAc/hexanes)	B
18	H	Cyclobutyl	H	2.06	216.09	Rf= 0.25 (30% EtOAc/hexanes)	B
19	H	3-Pentyl	H	2.55	232.11	Rf= 0.32 (30% EtOAc/hexanes)	B
20	Me	Phenyl	4-CN	1.96	277.10	Rf= 0.67 (EtOAc)	B
21	Me	Phenyl	4-NO_2	2.15	297.07	Rf= 0.69 (EtOAc)	B
22	Me	Phenyl	3-NO_2	2.11	297.07	Rf= 0.68 (EtOAc)	B
23	Me	Phenyl	4-Cl	2.40	286.05	Rf= 0.75 (EtOAc)	B
24	Me	Phenyl	2-Cl	2.25	286.08	Rf= 0.73 (EtOAc)	B

Ex. No.	R^3	A	R^2	t_R min [a,b]	MS[a,b] $(M+H)^+$	TLC R*f* (solvent)	General Method
25	Me	Phenyl	3,4-diCl	2.57	320.04	R*f*= 0.73 (EtOAc)	B
26	Me	Phenyl	4-Me	2.29	266.07	R*f*= 0.67 (EtOAc)	B
27	Me	Phenyl	4-Br	2.40	330.04	R*f*= 0.69 (EtOAc)	A, B
28	Me	CH_2-Phenyl	H	2.18	266.09	R*f*= 0.75 (EtOAc)	B
29	Me	Cyclo hexyl	H	2.46	258.17	R*f*= 0.75 (EtOAc)	B
30	Me	3-Pentyl	H	2.39	246.13	R*f*= 0.77 (EtOAc)	B

Table 1 Note a: HPLC - electrospray mass spectra (HPLC ES-MS) were obtained using a Hewlett-Packard 1100 HPLC equipped with a quaternary pump, a variable wavelength detector set at 254 nm, a YMC pro C-18 column (2 x 23 mm, 120A), and a Finnigan LCQ ion trap mass spectrometer with electrospray ionization. Spectra were scanned from 120-1200 amu using a variable ion time according to the number of ions in the source. The eluents were A: 2% acetonitrile in water with 0.02% TFA and B: 2% water in acetonitrile with 0.018% TFA. Gradient elution from 10% B to 95% over 3.5 minutes at a flowrate of 1.0 mL/min was used with an initial hold of 0.5 minutes and a final hold at 95% B of 0.5 minutes. Total run time was 6.5 minutes.

Table 1 Note b: Molecular ion data obtained via electrospray ionization.

The following Examples of 2-(substituted)-5-(3-pyridyl)tetrazole derivatives are prepared from General Intermediates H-L according to General Methods A and B:

Table 2. Examples 31 – 135 : Tetrazole Derivatives which may be Prepared from General Intermediates H-L by General Methods A and B.

Example No.	Compound Name
Example 31	5-(4-Ethyl-3-pyridyl)-2-(phenylmethyl)tetrazole
Example 32	5-(4-Ethyl-3-pyridyl)-2-((3-methoxyphenyl)methyl)tetrazole
Example 33	5-(4-Ethyl-3-pyridyl)-2-((2-methylphenyl)methyl)tetrazole
Example 34	5-(4-Ethyl-3-pyridyl)-2-((3-methylphenyl)methyl)tetrazole
Example 35	5-(4-Ethyl-3-pyridyl)-2-((4-fluorophenyl)methyl)tetrazole
Example 36	5-(4-Ethyl-3-pyridyl)-2-((2-bromophenyl)methyl)tetrazole
Example 37	5-(4-Ethyl-3-pyridyl)-2-((3-bromophenyl)methyl)tetrazole
Example 38	5-(4-Ethyl-3-pyridyl)-2-((3-nitrophenyl)methyl)tetrazole
Example 39	5-(4-Ethyl-3-pyridyl)-2-((4-nitrophenyl)methyl)tetrazole
Example 40	5-(4-Ethyl-3-pyridyl)-2-((2-chlorophenyl)methyl)tetrazole
Example 41	5-(4-Ethyl-3-pyridyl)-2-((3-chlorophenyl)methyl)tetrazole
Example 42	5-(4-Ethyl-3-pyridyl)-2-((4-chlorophenyl)methyl)tetrazole
Example 43	5-(4-Ethyl-3-pyridyl)-2-((4-methylphenyl)methyl)tetrazole
Example 44	5-(4-Ethyl-3-pyridyl)-2-((3,4-dichlorophenyl)methyl)tetrazole
Example 45	5-(4-Ethyl-3-pyridyl)-2-((2-nitrophenyl)methyl)tetrazole
Example 46	5-(4-Ethyl-3-pyridyl)-2-((2-napthyl)methyl)tetrazole
Example 47	5-(4-Ethyl-3-pyridyl)-2-((cyclohexyl)methyl)tetrazole
Example 48	5-(4-Ethyl-3-pyridyl)-2-((cyclobutyl)methyl)tetrazole
Example 49	5-(4-Ethyl-3-pyridyl)-2-((4-cyanophenyl)methyl)tetrazole
Example 50	5-(4-Ethyl-3-pyridyl)-2-(2-ethylbutyl)tetrazole
Example 51	5-(4-Ethyl-3-pyridyl)-2-((2-phenylethyl)tetrazole
Example 52	5-(4-(2-Propyl)-3-pyridyl)-2-(phenylmethyl)tetrazole
Example 53	5-(4-(2-Propyl)-3-pyridyl)-2-((3-methoxyphenyl)methyl)tetrazole
Example 54	5-(4-(2-Propyl)-3-pyridyl)-2-((2-methylphenyl)methyl)tetrazole
Example 55	5-(4-(2-Propyl)-3-pyridyl)-2-((3-methylphenyl)methyl)tetrazole
Example 56	5-(4-(2-Propyl)-3-pyridyl)-2-((4-fluorophenyl)methyl)tetrazole
Example 57	5-(4-(2-Propyl)-3-pyridyl)-2-((2-bromophenyl)methyl)tetrazole
Example 58	5-(4-(2-Propyl)-3-pyridyl)-2-((3-bromophenyl)methyl)tetrazole
Example 59	5-(4-(2-Propyl)-3-pyridyl)-2-((3-nitrophenyl)methyl)tetrazole
Example 60	5-(4-(2-Propyl)-3-pyridyl)-2-((4-nitrophenyl)methyl)tetrazole
Example 61	5-(4-(2-Propyl)-3-pyridyl)-2-((2-chlorophenyl)methyl)tetrazole

Example No.	Compound Name
Example 62	5-(4-(2-Propyl)-3-pyridyl)-2-((3-chlorophenyl)methyl)tetrazole
Example 63	5-(4-(2-Propyl)-3-pyridyl)-2-((4-chlorophenyl)methyl)tetrazole
Example 64	5-(4-(2-Propyl)-3-pyridyl)-2-((4-methylphenyl)methyl)tetrazole
Example 65	5-(4-(2-Propyl)-3-pyridyl)-2-((3,4-dichlorophenyl)methyl)tetrazole
Example 66	5-(4-(2-Propyl)-3-pyridyl)-2-((2-nitrophenyl)methyl)tetrazole
Example 67	5-(4-(2-Propyl)-3-pyridyl)-2-((2-napthyl)methyl)tetrazole
Example 68	5-(4-(2-Propyl)-3-pyridyl)-2-((cyclohexyl)methyl)tetrazole
Example 69	5-(4-(2-Propyl)-3-pyridyl)-2-((cyclobutyl)methyl)tetrazole
Example 70	5-(4-(2-Propyl)-3-pyridyl)-2-((4-cyanophenyl)methyl)tetrazole
Example 71	5-(4-(2-Propyl)-3-pyridyl)-2-(2-ethylbutyl)tetrazole
Example 72	5-(4-(2-Propyl)-3-pyridyl)-2-((2-phenylethyl)tetrazole
Example 73	5-(4-cyclopropyl-3-pyridyl)-2-(phenylmethyl)tetrazole
Example 74	5-(4-cyclopropyl-3-pyridyl)-2-((3-methoxyphenyl)methyl)tetrazole
Example 75	5-(4-cyclopropyl-3-pyridyl)-2-((2-methylphenyl)methyl)tetrazole
Example 76	5-(4-cyclopropyl-3-pyridyl)-2-((3-methylphenyl)methyl)tetrazole
Example 77	5-(4-cyclopropyl-3-pyridyl)-2-((4-fluorophenyl)methyl)tetrazole
Example 78	5-(4-cyclopropyl-3-pyridyl)-2-((2-bromophenyl)methyl)tetrazole
Example 79	5-(4-cyclopropyl-3-pyridyl)-2-((3-bromophenyl)methyl)tetrazole
Example 80	5-(4-cyclopropyl-3-pyridyl)-2-((3-nitrophenyl)methyl)tetrazole
Example 81	5-(4-cyclopropyl-3-pyridyl)-2-((4-nitrophenyl)methyl)tetrazole
Example 82	5-(4-cyclopropyl-3-pyridyl)-2-((2-chlorophenyl)methyl)tetrazole
Example 83	5-(4-cyclopropyl-3-pyridyl)-2-((3-chlorophenyl)methyl)tetrazole
Example 84	5-(4-cyclopropyl-3-pyridyl)-2-((4-chlorophenyl)methyl)tetrazole
Example 85	5-(4-cyclopropyl-3-pyridyl)-2-((4-methylphenyl)methyl)tetrazole
Example 86	5-(4-cyclopropyl-3-pyridyl)-2-((3,4-dichlorophenyl)methyl)tetrazole
Example 87	5-(4-cyclopropyl-3-pyridyl)-2-((2-nitrophenyl)methyl)tetrazole
Example 88	5-(4-cyclopropyl-3-pyridyl)-2-((2-napthyl)methyl)tetrazole
Example 89	5-(4-cyclopropyl-3-pyridyl)-2-((cyclohexyl)methyl)tetrazole
Example 90	5-(4-cyclopropyl-3-pyridyl)-2-((cyclobutyl)methyl)tetrazole
Example 91	5-(4-cyclopropyl-3-pyridyl)-2-((4-cyanophenyl)methyl)tetrazole
Example 92	5-(4-cyclopropyl-3-pyridyl)-2-(2-ethylbutyl)tetrazole

Example No.	Compound Name
Example 93	5-(4-cyclopropyl-3-pyridyl)-2-((2-phenylethyl)tetrazole
Example 94	5-(4-cyclopentyl-3-pyridyl)-2-(phenylmethyl)tetrazole
Example 95	5-(4-cyclopentyl-3-pyridyl)-2-((3-methoxyphenyl)methyl)tetrazole
Example 96	5-(4-cyclopentyl-3-pyridyl)-2-((2-methylphenyl)methyl)tetrazole
Example 97	5-(4-cyclopentyl-3-pyridyl)-2-((3-methylphenyl)methyl)tetrazole
Example 98	5-(4-cyclopentyl-3-pyridyl)-2-((4-fluorophenyl)methyl)tetrazole
Example 99	5-(4-cyclopentyl-3-pyridyl)-2-((2-bromophenyl)methyl)tetrazole
Example 100	5-(4-cyclopentyl-3-pyridyl)-2-((3-bromophenyl)methyl)tetrazole
Example 101	5-(4-cyclopentyl-3-pyridyl)-2-((3-nitrophenyl)methyl)tetrazole
Example 102	5-(4-cyclopentyl-3-pyridyl)-2-((4-nitrophenyl)methyl)tetrazole
Example 103	5-(4-cyclopentyl-3-pyridyl)-2-((2-chlorophenyl)methyl)tetrazole
Example 104	5-(4-cyclopentyl-3-pyridyl)-2-((3-chlorophenyl)methyl)tetrazole
Example 105	5-(4-cyclopentyl-3-pyridyl)-2-((4-chlorophenyl)methyl)tetrazole
Example 106	5-(4-cyclopentyl-3-pyridyl)-2-((4-methylphenyl)methyl)tetrazole
Example 107	5-(4-cyclopentyl-3-pyridyl)-2-((3,4-dichlorophenyl)methyl)tetrazole
Example 108	5-(4-cyclopentyl-3-pyridyl)-2-((2-nitrophenyl)methyl)tetrazole
Example 109	5-(4-cyclopentyl-3-pyridyl)-2-((2-napthyl)methyl)tetrazole
Example 110	5-(4-cyclopentyl-3-pyridyl)-2-((cyclohexyl)methyl)tetrazole
Example 111	5-(4-cyclopentyl-3-pyridyl)-2-((cyclobutyl)methyl)tetrazole
Example 112	5-(4-cyclopentyl-3-pyridyl)-2-((4-cyanophenyl)methyl)tetrazole
Example 113	5-(4-cyclopentyl-3-pyridyl)-2-(2-ethylbutyl)tetrazole
Example 114	5-(4-cyclopentyl-3-pyridyl)-2-((2-phenylethyl)tetrazole
Example 115	5-(4-phenyl-3-pyridyl)-2-(phenylmethyl)tetrazole
Example 116	5-(4-phenyl-3-pyridyl)-2-((3-methoxyphenyl)methyl)tetrazole
Example 117	5-(4-phenyl-3-pyridyl)-2-((2-methylphenyl)methyl)tetrazole
Example 118	5-(4-phenyl-3-pyridyl)-2-((3-methylphenyl)methyl)tetrazole
Example 119	5-(4-phenyl-3-pyridyl)-2-((4-fluorophenyl)methyl)tetrazole
Example 120	5-(4-phenyl-3-pyridyl)-2-((2-bromophenyl)methyl)tetrazole
Example 121	5-(4-phenyl-3-pyridyl)-2-((3-bromophenyl)methyl)tetrazole
Example 122	5-(4-phenyl-3-pyridyl)-2-((3-nitrophenyl)methyl)tetrazole
Example 123	5-(4-phenyl-3-pyridyl)-2-((4-nitrophenyl)methyl)tetrazole

Example No.	Compound Name
Example 124	5-(4-phenyl-3-pyridyl)-2-((2-chlorophenyl)methyl)tetrazole
Example 125	5-(4-phenyl-3-pyridyl)-2-((3-chlorophenyl)methyl)tetrazole
Example 126	5-(4-phenyl-3-pyridyl)-2-((4-chlorophenyl)methyl)tetrazole
Example 127	5-(4-phenyl-3-pyridyl)-2-((4-methylphenyl)methyl)tetrazole
Example 128	5-(4-phenyl-3-pyridyl)-2-((3,4-dichlorophenyl)methyl)tetrazole
Example 129	5-(4-phenyl-3-pyridyl)-2-((2-nitrophenyl)methyl)tetrazole
Example 130	5-(4-phenyl-3-pyridyl)-2-((2-napthyl)methyl)tetrazole
Example 131	5-(4-phenyl-3-pyridyl)-2-((cyclohexyl)methyl)tetrazole
Example 132	5-(4-phenyl-3-pyridyl)-2-((cyclobutyl)methyl)tetrazole
Example 133	5-(4-phenyl-3-pyridyl)-2-((4-cyanophenyl)methyl)tetrazole
Example 134	5-(4-phenyl-3-pyridyl)-2-(2-ethylbutyl)tetrazole
Example 135	5-(4-phenyl-3-pyridyl)-2-((2-phenylethyl)tetrazole

Determination of the activity of the compounds of the invention

C17,20 Lyase inhibitory activity of compounds can be determined using, e.g., the biochemical or the cellular assays set forth in the Examples. A person of skill in the art will recognize that variants of these assays can also be used.

The compounds of the invention can also be tested in animal models, e.g., animal models of prostate or breast cancer.

Each of the compounds of the invention was subjected to a biochemical assay and a cellular assay for determining its C17,20 lyase inhibitory activity.

Human and murine C17,20 lyase biochemical assays:

Recombinant human C17,20 lyase (hLyase) was expressed in (Sf9) cells, and hLyase enriched microsomes were prepared from cultures as described in the following reference: Baculovirus Expression of Bovine P_{450} in Sf9 Cells and Comparison with Expression in Yeast, Mammalian Cells, and *E. Coli*. Barnes H. J.; Jenkins, C. M.; Waterman, M. R., *Archives of Biochemistry and Biophysics* (1994) 315(2) 489-494. Recombinant murine C17,20 lyase (mLyase) was prepared in a similar manner. hLyase and mLyase preparations were titrated using assay conditions to determine protein concentrations to be used for

assays. Both mLyase and hLyase assays were run in an identical manner except that cytochrome b5 was omitted in the murine assays.

Test compounds were diluted 1:4, serially in six steps, with 100% DMSO starting from 800 μM going to 51.2 nM reserving the first 2 columns for the generation of a standard curve. Each of these compound solutions in 100% DMSO was further diluted twenty fold in H_2O to obtain compound concentrations ranging from 40 μM to 2.56 nM in 5% DMSO. Dehydroepiandrosterone (DHEA) standards were serially diluted in 100% DMSO from 400 μM down to 120 nM in half-log dilutions. Each dilution was further diluted twenty fold in H_2O to obtain 20 μM to 6 nM solutions in 5% DMSO using the first 2 columns. Five μl of these 5% DMSO dilutions were used in the assay.

Clear-bottomed opaque 96 well assay plates were loaded with 50 μL of assay buffer (50 mM Na_3PO_4, pH 7.5) and 5 μL of the diluted compounds were added to the wells. Thirty μL of substrate solution (7 mM NADPH (Sigma N1630), 3.35 μM 17-OH-pregnenolone (Steraloids Q4710), 3.35 μg/mL human cytochrome b_5 (Panvera P2252) in 50 mM sodium phosphate pH 7.5 buffer) was added to all wells. Reactions were initiated with the addition of 10 μL hLyase or mLyase in assay buffer.

Enzymatic reactions were allowed to run for 2 hours at room temperature with gentle agitation. Reactions were terminated with the addition of 50 μM (final concentration) YM116, a potent C17,20 lyase inhibitor. The concentration of DHEA generated by hLyase was determined by radioimmunoassay (RIA) as described below.

0.08 μCi ^{3}H-DHEA (1.6 μCi/mL) (NEN (NET814)) in scintillation proximity assay (SPA) buffer (100 mM Tris-HCl, pH 7.5, 50 mM NaCl, 0.5% BSA (Sigma A9647), 0.2% Tween 20) was added to each well. Fifty μL DHEA rabbit antiserum with anti-rabbit SPA beads in SPA buffer was added to all wells. Anti DHEA rabbit antiserum was obtained from Endocrine Sciences (D7-421) (1 mL H_2O to the vial) and anti-Rabbit SPA Beads were obtained from Amersham (RPNQ 0016) (6mL SPA buffer to the bottle). Mixtures were allowed to equilibrate with gentle agitation for 1 hour followed by an overnight equilibration with no agitation. ^{3}H-DHEA bound to the SPA beads was determined by scintillation counting.

The concentration of DHEA generated in each reaction was calculated from raw data (CPM) and the standard curve. The lyase inhibitory activity of each compound was

determined as the concentration of DHEA generated in the presence of test compounds, expressed as a percent inhibition compared to the DHEA concentration generated in the absence of test compounds (1-(nM DHEA formed in the presence of test compound/nM DHEA formed in the absence of test compounds) x 100).

Human C17,20 cellular assay:

Human 293 lyase cells were prepared as described above for the Sf9 cells [Baculovirus Expression of Bovine Cytochrome P_{450} in Sf9 Cells and Comparison with Expression in Yeast, Mammalian Cells, and *E.Coli*. Barnes, H. J.; Jenkins, C. M.; Waterman, M. R. *Archives of Biochemistry and Biophysics* (1994) 315 (2) 489-494]. The cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) /10% FBS/ 1%S/P/1%L-Glu/0.8mg/mLG418/HEPES.

On day one, human 293 lyase cells were plated at 10,000 cells/well/100μL in columns 2-12 of a 96-well tissue culture plate (Falcon 3075), and allowed to attach overnight (each mother plate needs two cell plates).

On day two, 100 μL H_2O was added to all the wells of a daughter plate (one mother plate one daughter plate Costar 3365). DHEA standard was diluted with RPMI (4.5 μL of 500 μM into 3 mL RPMI, then 1:3 serial dilutions). The media from columns 2-12 of the cell plate was removed and replaced with 100 μL RPMI without phenol red. Diluted DHEA standards (100 μL) at a concentration of 750, 250, 83.3, 27.7, 9.2, 3, 1 and 0.3 nM were added to column 1 of the cell plate. 50 μL of 100% DMSO was added to columns 1 and 2 of the mother plate. 5 μL of compound was transferred from mother plate to daughter plate, then from the daughter plate to a cell plate using a robot. The cell plate was incubated for 10 minutes at room temperature. 15 μL of 10 mM 17-OH-pregnenolone (Steraloids (Q4710) (10 mM stock in 100% DMSO)) was diluted in 30 mL RPMI to obtain a solution of 5 μM 17-OH-pregnenolone. 10 μL of this solution was added to all the wells of the cell plate, except that column received only DMSO. The plate was then incubated for one hour at 37 °C.

The amount of DHEA produced was determined as follows. 90 μL media was removed from each well of the cell plate and placed into an SPA assay plate (Wallac Isoplate #1450). 50 μL of ^{3}H-DHEA (1.6 μCi/mL, New England Nuclear (Catalog # NET814)) was

added to each well of the SPA assay plate. 50 μL of anti-DHEA/anti-rabbit SPA beads (20 μL/mL AB with 10 mg/mL SPA beads) were then added to each well of the plate. The plate was incubated overnight, and the radioactivity counted as described above. The first two columns of the plate were reserved for a standard curve of DHEA and the no compound controls.

The raw data (CPM) was converted to a concentration of DHEA formed (nM) by use of the standard curve. The lyase inhibitory activity of the compounds was determined as the amount of DHEA formed in the presence of compound compared to the amount formed in the absence of compound in the form of a percent inhibition (1- (nM DHEA formed with compound/nM DHEA formed without compound) x 100).

A test compound was considered to be active if the IC_{50} in the human C17,20 biochemical assay or in the human C17,20 cellular assay was less than 10 μM. All the compounds tested have IC_{50} in the human C17,20 biochemical assay or the human C17,20 cellular assay of less than 10 μM.

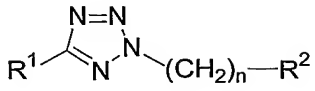
Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

CLAIMS

We claim

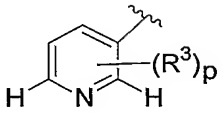
1. A compound of the formula



wherein

n is 1 or 2;

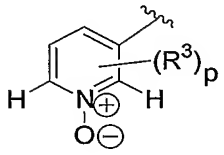
R^1 represents



wherein

R^3 is C_{1-4} alkyl or C_{3-5} cycloalkyl; and

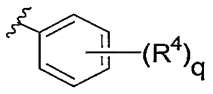
p is 0, 1, or 2; or



, provided that R^2 is other than a pyridyl group;

and

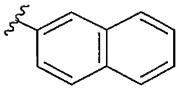
R^2 represents



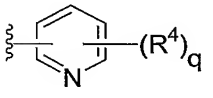
wherein

R^4 represents C_{1-4} alkyl, C_{1-4} alkoxy, C_{3-6} cycloalkyl, halogen, NO_2, or CN; and

q is 0, 1, or 2;



; or



;

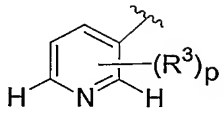
or a pharmaceutically acceptable salt thereof.

2. A compound according to claim 1

wherein

n is 1 or 2;

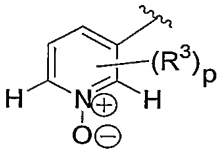
R^1 represents



wherein

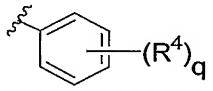
R^3 is C_{1-4} alkyl or C_{3-5} cycloalkyl; and

p is 0, 1, or 2; or



and

R^2 represents



wherein

R^4 represents C_{1-4} alkyl, C_{1-4} alkoxy, halogen, NO_2, or CN; and

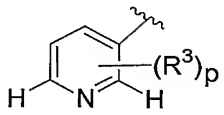
q is 0, 1, or 2.

3. A compound according to claim 1

wherein

n is 1;

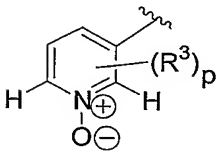
R^1 represents



wherein

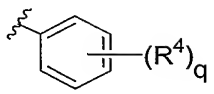
R^3 is C_{1-4} alkyl or C_{3-5} cycloalkyl; and

p is 0, 1, or 2; or



and

R^2 represents



wherein

R^4 represents C_{1-4} alkoxy, halogen, or NO_2; and

q is 0, 1, or 2.

4. A pharmaceutical composition comprising a compound of claim 1 and a pharmaceutically acceptable carrier.

5. A method of inhibiting a lyase enzyme, comprising contacting said lyase enzyme with a compound of claim 1.

6. A method of inhibiting a 17α-hydroxylase-C17,20 lyase, comprising contacting a 17α-hydroxylase-C17,20 lyase with a compound of claim 1.

7. A method for treating a subject having a cancer associated with a 17α-hydroxylase-C17,20 lyase, comprising administering to the subject a therapeutically effective amount of a compound of claim 1.

8. A method for treating prostate cancer in a subject, comprising administering to said subject a therapeutically effective amount of a compound of claim 1, such that the prostate cancer in the subject is treated.

9. A method for treating breast cancer in a subject, comprising administering to said subject a therapeutically effective amount of a compound of claim 1, such that the breast cancer in the subject is treated.

10. The method of any one of claims 7-9, wherein said subject is a primate, equine, canine or feline.

11. The method of any one of claims 7-9, wherein said subject is a human.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 02/30982

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07D401/04 A61K31/4439 A61P35/00 //(C07D401/04,257:00, 213:00)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07D A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, INSPEC, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE CA 'Online! CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US; ISHMETOVA, R. I. ET AL: "Ultrasonic alkylation of 5-substituted 1H-tetrazoles by Mannich bases" retrieved from STN Database accession no. 120:217462 XP002226378 RN 104186-22-3 abstract & KHIMIYA GETEROTSIKLICHESKIKH SOEDINENII (1993), (8), 1060-3 , --- -/--	1

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
7 January 2003	21/01/2003

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Goss, I

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 02/30982

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